HEAVY METALS
IN WATER ORGANISMS

Symposia Biologica Hungarica 29

HEAVY METALS IN WATER ORGANISMS

29

Akadémiai Kiadó, Budapest

HEAVY METALS IN WATER ORGANISMS

Edited by
J. SALÁNKI

(Symposia Biologica Hungarica 29.)

Chemical substances of anthropogenic origin polluting surface waters are dangerous to aquatic organisms and have varying effects on microbes, plants and animals. Living organisms frequently accumulate pollutants, signalling in this way their presence in the environment. Accumulation is particularly characteristic of heavy metals entering surface waters as industrial, agricultural and communal wastes.

Heavy metals have come into the focus of environmental biological research all over the world. Beyond the monitoring of pollution, special attention is being paid to the various organisms which make early detection possible.

The papers in this volume were presented at a symposium held in Hungary at which scientists from 12 countries provided information on the latest results in this field. The most important topics discussed were accumulation of heavy metals, ecological monitoring of heavy metal pollution, organisms as indicators and the effect of heavy metal pollution on vital functions. The Hungarian participants gave an account of the state-of-the-art of research into the heavy metal pollution of Lake Balaton for the first time.

The seriousness of the problem of heavy metal pollution is reflected by the fact that it has assumed prominence in one of the main projects of IUBS.

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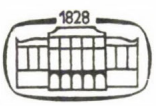
Director of the Balaton Limnological
Research Institute of the Hungarian
Academy of Sciences, Tihany, Hungary
and
Vice-President of the International
Union of Biological Sciences



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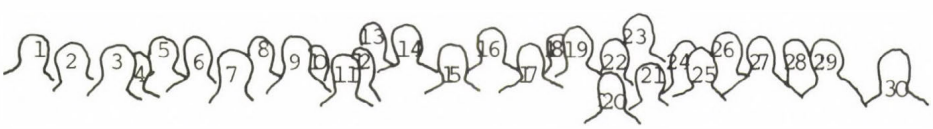

PREFACE

The papers of the Volume were presented at a Symposium organized by the Balaton Limnological Research Institute of the Hungarian Academy of Sciences, between 1 and 7 September, 1984.

In spite of the larga number of publications which have appeared in the field, the topic is most timely since heavy metal pollution constitutes a major part of environmental problems. Heavy metals of various origin may pollute surface waters causing damage to aquatic organisms and their consumers. The Symposium was organized to provide an opportunity for discussing recent results in different countries and institutes.

The host institute located at Lake Balaton, was established on the Tihany peninsula in 1927. Its name was, until recently, Biological Research Institute of the Hungarian Academy of Sciences. The change of the name to Balaton Limnological Research Institute reflects the fact that a great deal of research is directed at questions on the biology of the Lake, which is also endangered by environmental influences. The papers presented in this Volume from the Institute represent various approaches necessary for understanding the fate and effect of heavy metals in complex biological systems.

Special thanks are due to colleagues who helped to organize the Symposium and to prepare this Volume, especially to Dr. László Hernádi, Dr. Maria Kiss and to Judith Komáromi. Their enthusiasm and assistance were a great help to me.

The Editor

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OPENING ADDRESS

J. SALÁNKI

Director of the Balaton Limnological Research Institute
of the Hungarian Academy of Sciences, Tihany
and
Vice-President of IUBS

Ladies and Gentlemen.

It is a great pleasure to greet you at the opening of the Symposium on Heavy Metals in Water Organisms at this picturesque part of Lake Balaton. The organization of this Symposium was motivated by several factors. One of these was that, in spite of serious demands and measures taken against contamination of the environment with heavy metals, very little or no progress has been achieved in this respect and the aquatic organisms in lakes, rivers and in the sea are under the constant impact of earlier and renewed metal pollution. The second reason was that more attention has been devoted to the organisms which are able to indicate low levels of toxic metal pollution in natural waters; therefore, they can be recommended in practical application as bioindicator organisms. At this symposium we wish to create an opportunity for discussion and presentation of the latest results and exchange of experiences on these topics.

Accumulation studies and selection of best accumulator species in aquatic ecosystems are only one aspect of the problem. Toxic doses of heavy metals causing serious deterioration or loss of life among aquatic animals is rather rare these days. However, the effect of low, sublethal concentrations may also cause serious damage in the tissues, organs and life processes of plants, microbes and animals. Such effects jeopardize various functions, biochemical processes, adaptation, feeding and reproduction in the long run. Moreover, heavy metal accumula-

tion can cause pathological changes; however, they can induce protective mechanisms which can be useful for the organism. When the call for papers to this symposium went out, we hoped to hear of results on the functional aspects of heavy metal pollution and I think that in the future we should strengthen the cooperation and understanding among those who are monitoring accumulators in nature and who are studying the effects and mechanisms of low-level pollution in the laboratory.

This Symposium has been sponsored by the Hungarian Academy of Sciences and by the International Union of Biological Sciences. I would like to explain just briefly why. The Hungarian Academy of Sciences supports a research project on Lake Balaton focussung on the effect of human impacts on the Lake. One of the main topics is investigation of heavy metal pollution of the water and its living organisms, especially plants and animals. Balaton is the largest shallow lake in Europe, its surface is about 600 km^2 but its depth is only 3 metres on an average. In the catchment area which is about 6000 km^2 there is no major industry, but agriculture is rather intensive, and in summer it is very popular with tourists. The main problem with the Lake is the increase of eutrophication and, to a lesser degree, contamination with toxic substances, among them heavy metals. Earlier, no survey had been made of heavy metal contamination of the Lake and that is why our Academy and also the Environmental Protection Agency support this kind of research by the Balaton Limnological Research Institute and other institutions.

The International Union of Biological Sciences is also interested in problems of heavy metal contamination in aquatic organisms. At the last General Assembly of the Union in 1982, a scientific programme was adopted for studying bioindicators of environmental pollution. One type of pollutant under study is heavy metals and a field of interest is water ecosystems. The general objectives of the programme are:

- to encourage scientists, as well as scientific and national bodies, to develop and improve methods indicative of hazardous substances occurring in the environment;

- to collect information about existing methods on bio-indicators;
- to promote exchange of experiences between different laboratories, to help dissemination of recent knowledge in different countries;
- to provide literature on bioindicators including different reference lists, general description to organizations concerned;
- to promote interdisciplinary and international cooperation in standardizing and extending the use of mutually accepted methods;
- to stimulate scientific bodies to encourage the presentation of new results concerned with bioindicators at international meetings;
- to organize special regional or international symposia, workshops and seminars in cooperation with national bodies or other organizations on the methods, new results and their applications.

Biologists of over 30 countries are cooperating in this project, which includes microbiology, botany, zoology, hydrobiology, comparative physiology and biochemistry, cell biology and genetics, i.e. practically all aspects of biology. A wide variety of anthropogenic substances are of interest, in fact, all chemicals which are able to cause damage in the environment. Both the accumulation and the fate of these substances in living systems are considered by the project to be also minor toxic effects and the response of organisms to low levels of contamination. Emphasis is focussed on the selection of bio-indicator organisms and functions which could be useful as warning systems in early elimination of harmful substances from the environment. To fulfil this project, IUBS has organized meetings and prompted the publication of results on bioindicators and the present meeting is one of these events.

We hope that the papers to be presented at this Symposium will come up to these expectations and that, by publishing the manuscripts and the discussions, we can give a wide publicity to the results we will talk about during the next few days.

I happen to be responsible for the bioindicator programme of IUBS and I also represent here the Hungarian Academy of Sciences. Therefore, on behalf of both of them, I would like to welcome you once again, and wish you fruitful discussions in, and outside, the lecture hall, and hope you have a very pleasant stay here at Balatonfüred and in Hungary, too.

ACCUMULATION OF HEAVY METALS

Symposia Biologica Hungarica 29 (1985)

THE UPTAKE OF SELECTED HEAVY METALS BY THE GREEN ALGA *CLADOPHORA GLOMERATA*

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INTRODUCTION

In terrestrial plant ecosystems both physiological and ecological aspects of metal uptake have been widely researched. Aquatic macrophytes are able to accumulate heavy metals to very high levels but fewer studies have been made for aquatic environments. In the last ten years or so, heavy metals have become an increasingly common contaminant of sea and freshwater. Lakes and their sediments have long been recognised as common sinks for metals. Rivers, similarly are capable of transporting large quantities of metals and many are used for water supply purposes.

Some rivers subjected to extremely high influxes of heavy metals as a result of past mining activities have been thoroughly investigated with regard to metal uptake by aquatic macrophytes (McLean, 1975; McLean and Jones, 1975) and in these environments metal resistance has been studied (McLean, 1975; Harding and Whitton, 1976; Foster, 1977; Say et al., 1977).

Far less work has been carried out into the effects of heavy metals in road and urban runoff on aquatic macrophytes in a moderately polluted urban river ecosystem and on the way in which macrophytes in these systems can be used as biological monitors of specific metals. Motorway and road runoff appears to be an important source of heavy metals to streams and rivers (Hedley and Lockley, 1975; Ellis, 1976; Laxen and Harrison, 1977; Revitt and Ellis, 1980) particularly lead and zinc.

The main aim of this study was to examine the uptake of metals by a primary producer *Cladophora glomerata* in an urban river receiving motorway, urban and non-point runoff.

Previous surveys have considered metal levels in *Cladophora glomerata* in lentic waters(Adams and Stone, 1973; Taft and Kischler, 1973; Keeney et al., 1976; Trollope and Evans, 1976) and lotic environments polluted by mining activities or industrial effluents (Gale et al., 1973; Adams et al., 1980).

The results submitted in this paper form part of a ten year programme on the effects of motorway and urban runoff on the macrophyte and inver communities in an urban river (Extence, 1978; Stone, 1981; Davis, 1984).

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MATERIALS AND METHODS

Study area

The river Roding rises near Molehill Green, Essex, England and flows for 82 km. entering the river Thames at Barking Creek (Fig.1). Sampling sites were selected from the middle reaches and the locations are given in Table 1.

Each sampling site comprised a 10m stretch of river bed ranging from 7.5-10m in width. As far as possible sites were chosen in riffle sections where current velocity, depth and substrate (coarse gravel 10-150mm) were similar.

Site 1 was selected as a 'control' site since here the river flowed across arable farm land and was remote from urban influences and sources of pollution. Sites 2 to 7 were all situated in the urban zone. Major environmental influences on each site are listed in Table 1 and major likely sources of metallic input into the river are illustrated diagrammatically in Fig. 2.

In addition to the seven sites, four inputs (runoffs) to the Roding were

Fig. 1. The river Roding and its major tributaries showing the position of sampling sites 1 to 7.

Table 1. Locations of sites sampled on the river Roding with environmental details

Site	Location	Grid Ref.	Environmental details
1	Abridge	TQ486 975	Crosses arable farmland. Small sewage works discharging good quality effluents into receiving tributaries upstream.
2	Debden (near junction 5 of M11)	TQ441 956	M11 motorway to the east. Recreational land to the west. Poor quality sewage effluent entering the river via tributaries. Debden Industrial estate upstream. Urban runoff draining Debden housing estate.
3	Buckhurst Hill recreation ground	TQ433 949	Large recreational area bordered by housing estate to the west. Open drainage channel (RO1) carrying runoff from area of motorway service station construction to the east.
4	Buckhurst Hill Lower Queen's Rd.	TQ424 936	Private land used for grazing horses.
5	Chigwell Luxborough Lane	TQ422 932	M11 motorway 0.5 km to the east. Buckhurst Hill drain (RO2) composed of urban runoff enters the river here.
6	"	"	Chigwell Brook which carries runoff from the M11 motorway and drains the urban area of Chigwell merges here.
7	"	TQ423 929	Just downstream of site 6 in a riffle section.

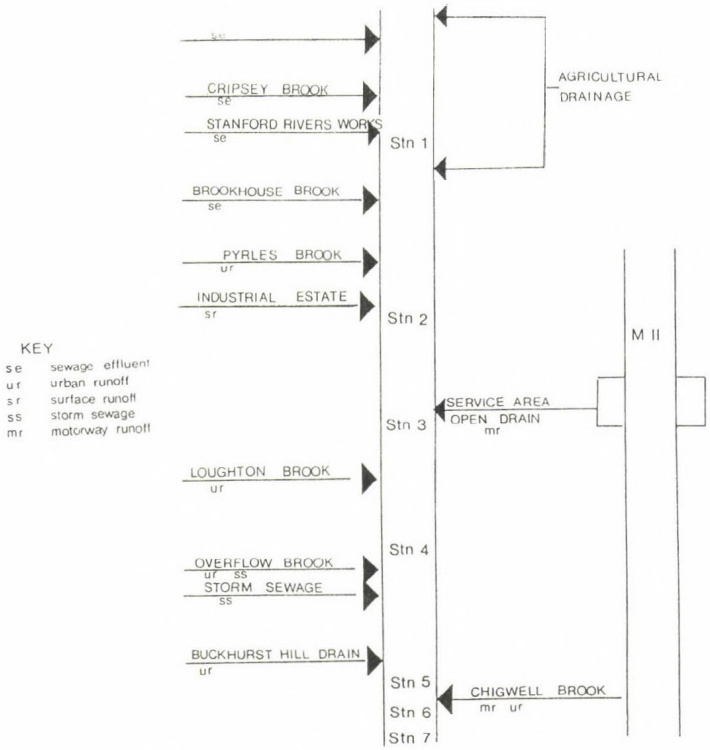

Fig. 2. Diagrammatic summary of potential sources of metallic pollution to the river Roding.

Table 2. Locations and descriptions of inputs to the river Roding

Input	Name	Description	Location
RO1		Open drainage channel draining area of service station construction	Joins river 40m above site 3 (90m following diversion in August 1977)
RO2	Buckhurst Hill drain	Covered surface water drain carrying urban runoff	Enters river 20m upstream of site 5 via a culvert on west bank
RO3c	Chigwell Brook	Carries runoff from M11 and urban area of Chigwell	Just upstream of site 6 entering river via east bank
RO3b	Chigwell Brook		At point where it emerges from under the M11
RO3a		M11 runoff drain	Enters Chigwell Brook at RO3b

sampled for metals and these are listed in Table 2.

The M11 motorway which runs from Redbridge in the south to Cambridge in the north became operational in April 1977, four months after the commencement of this study. It runs parallel with the river for many kilometres in the urban zone and also traverses it in places.

Field collections

The green alga *Cladophora glomerata* was collected from sites 1, 2, 3, 4, and 7 at 3-4 weekly intervals from January 1977 to May 1979. Collection was subject to availability and condition of the plant and the number of collections made ranged from eleven at site 3 to twenty at site 2. The alga was additionally collected from inputs RO2, RO3a and RO3b on selected dates.

Plants were always collected at times of low flow to ensure representative samples. Filaments were less likely to be clogged with silt at this time thus facilitating the cleaning process. Sampling was restricted as far as possible to plants growing in or near the main current in mid-channel. Healthy-looking specimens were selected avoiding very young or very silted plants. At least three replicate samples were taken at each site and in some instances as many as ten. Samples were placed in small polythene bags and dealt with on return to the laboratory. The plants were thoroughly washed to remove foreign matter and then rinsed three times with distilled water and blotted dry.

Samples were placed in acid washed evaporating dishes and dried for at least three days at 60-70^{o}C in an oven. An acid resistant pestle and mortar was used to grind samples to a powder fine enough to pass through a 0.5-0.7mm sieve. The samples were then transferred to air tight specimen tubes. Before weighing out suitable amounts for acid digestion, samples were dried at 105^{o}C to constant weight and then desiccated.

Water samples were collected in mid-stream about 10cm below the surface in 500cm^{3} polythene bottles which had been previously soaked in dilute nitric acid and rinsed with distilled water several times.

Laboratory analyses

Plant and water samples were analysed for lead, cadmium, copper, nickel, zinc, manganese and iron. Subsamples of homogenous ground plant tissue were digested in concentrated 'Analar' nitric acid. Desiccated dried samples of 0.5g weight were digested with 20cm^{3} acid in 50cm^{3} pyrex flasks covered by glass spheres. The digestions were run at 80^{o}C until digestion was complete (about three days). The digests were evaporated just to dryness and the residues taken up in 0.01M hydrochloric acid to standard volume.

Water samples were prepared for total metals analyses by concentrating a suitable volume of water then digesting it with 5cm^{3} 50% v/v 'Analar' nitric acid and 0.5cm^{3} 30% hydrogen peroxide. Lanthanum chloride was added as an interference suppressant.

Metal analyses were made using a Perkin-Elmer 272 atomic absorption spectrophotometer. An air-acetylene flame was used for all metals and basic operating conditions recommended by the manufacturer were followed (Perkin-Elmer,1976). Matrix interferences were measured and assimilated by use of

a built-in deuterium arc background corrector facility.

RESULTS

Water chemistry

Concentrations of metals in water samples from Roding sites and selected inputs are given in Tables 3 and 4. The Kruskal-Wallis and Mann-Whitney U tests were used to analyse spatial differences. A full description of chemical data at these particular sites and inputs is given by the author (Stone, 1981).

Concentrations of total lead, copper and manganese were generally lower at site 1, the control site (significantly lower with regard to copper and manganese). A marked increase occurred at site 2 indicating a metallic input above this site with copper concentrations peaking here. Lead, nickel and zinc continued to increase downstream of the control site and achieved maximum mean levels at site 3. Site 4 displayed a decline in metal levels but this process was soon reversed a few kilometres downstream at sites 5 and 6 with iron peaking at the latter.

Table 4 indicates that the open drainage channel RO1, the Buckhurst Hill drain urban runoff(RO2) and the Chigwell Brook RO3b and RO3c (motorway and urban runoff) at times contributed high concentrations of metals to the river at sites 3,5 and 6 respectively. RO1 was certainly responsible at times for elevated lead, copper, cadmium, nickel and iron at site 3. Mean values were generally very similar to those obtained for river sites reflecting the intermittent nature of the problem.

Cladophora glomerata

Mean coefficients of variation for metals in algal tissue are given in Table 5. They give an idea of within site variation. Variability was particularly high for cadmium (46.3%).

Mean metal concentrations in *Cladophora* along with range of values are listed in Table 6. Spatial comparisons have been made using one-way analysis of variance and significance levels are shown.

Metals were present in higher concentrations in algal tissue than in the water itself. Statistically significant differences between sites were obtained for only three of the metals;- zinc($F_{4,70}=2.68$, $p=0.05$), lead ($F_{4,72}=3.83$, $p=0.01$) and iron($F_{4,57}=4.04$, $p=0.01$).

Maximum mean concentrations of lead (58.8 $\mu g\ g^{-1}$), copper(35.9), zinc (193) and iron(8519) were recorded at site 4 whilst cadmium (1.70) peaked at site 3 and nickel (23.0) and manganese (1376) achieved maximum means at site 7.

With the exception of nickel, mean metal content of the alga at site 1 was generally lower than at sites downstream. Lead and manganese were almost twice as high at downstream locations.

Fig. 3 further depicts the trends graphically and shows the relationship of *Cladophora* metals with water metals. Mean metal content of the alga showed similar downstream trends to that of the water itself. Zinc, lead and copper increased notably downstream at site 2 as did the same metals

Table 3. Mean concentrations of total metals ($\mu g\ dm^{-3}$ ± standard error of the mean) in water samples from sites on the river Roding 1977-1979; Ranges are given

Site	pH	Pb	Cd	Cu	Ni	Zn	Fe	Mn
1	7.6-9.1	37 ± 3 (10-80)	23 ± 14 (N/D-490)	16 ± 2 $ (7-28)	28 ± 2 (N/D-70)	51 ± 6 (13-174)	800 ± 320 (120-10500)	40 ± 9* (10-300)
2	7.4-8.8	41 ± 4 (10-100)	8 ± 1 (2-30)	27 ± 5 (8-130)	29 ± 3 (10-60)	64 ± 9 (20-236)	1080 ± 460 (140-12200)	59 ± 9 (20-230)
3	7.3-9.1	53 ± 7 (10-210)	17 ± 6 (2-130)	23 ± 4 (8-123)	34 ± 4 (N/D-100)	96 ± 23 (17-700)	920 ± 300 (130-9000)	56 ± 8 (10-240)
4	7.3-8.9	37 ± 3 (13-60)	7 ± 1 (3-20)	23 ± 5 (7-140)	27 ± 2 (14-50)	52 ± 6 (18-138)	650 ± 250 (90-7400)	50 ± 7 (20-210)
5	7.4-8.9	50 ± 7 (14-210)	9 ± 2 (3-30)	22 ± 3 (8-200)	31 ± 3 (10-80)	74 ± 12 (17-310)	840 ± 290 (110-9500)	62 ± 9 (10-230)
6	7.4-8.9	44 ± 7 (14-210)	8 ± 1 (2-40)	21 ± 2 (10-60)	30 ± 3 (10-100)	64 ± 8 (170-260)	1330 ± 480 (N/D-15400)	60 ± 8 (20-210)

* Significantly lower than sites 2 to 6
$ Significantly lower than sites 2,5 and 6
N/D Non-detectable

Table 4. Mean concentrations of total metals (µg dm^{-3} ± standard error of the mean) in water samples from selected inputs into the river Roding 1977-1979; Ranges are given

Input	pH	Pb	Cd	Cu	Ni	Zn	Fe	Mn
RO1	6.8-10.0	50 ± 6 (N/D-104)	8 ± 1 (N/D-20)	41 ± 21 (N/D-370)	30 ± 3 (10-60)	79 ± 13 (20-193)	2660 ± 1250 (180-20000)	11 ± 26 (30-410)
RO2	7.7-8.2	38 ± 12 (N/D-430)	7 ± 1 (N/D-20)	22 ± 2 (N/D-50)	37 ± 3 (N/D-80)	135 ± 15 (38-320)	470 ± 160 (60-5000)	176 ± 19 (40-460)
RO3c	7.2-9.4	32 ± 8 (10-210)	9 ± 3 (2-90)	24 ± 3 (N/D-80)	41 ± 5 (N/D-170)	64 ± 9 (180-187)	2360 ± 670 (30-20400)	107 ± 8 (40-250)
RO3b	7.6-8.5	N.S	5 ± 0 (2-7)	24 ± 4 (8-60)	25 ± 3 (12-44)	77 ± 18 (24-217)	4630 ± 2080 (340-30200)	110 ± 25 (27-320)

N/D Non-detectable
N.S No sample

Table 5. Mean coefficients of variation (C.V) for *Cladophora* metal content at all sites 1977-1979

Mean C.V.	Pb	Cd	Cu	Ni	Zn	Fe	Mn
%	21.6	46.3	18.7	23.9	14.9	24.3	22.9

Table 6. Mean concentrations of metals(µg g^{-1} dry weight + standard error of the mean) in *Cladophora glomerata* from sites on the river Roding 1977-79; Ranges are given.

	1	2	3	4	7
Pb	*28.9(3.0)	49.1(4.3)	51.6(8.5)	58.8(4.4)	56.3(7.2)
	10.8-54.1	17.2-78.9	15.5-103	32.2-114	25.6-150
Cd	0.089(0.14)	1.06(0.16)	1.70(0.35)	1.62(0.23)	1.24(0.22)
	0.24-1.69	0.19-1.99	0.32-3.49	0.65-4.03	0.25-3.05
Ni	21.3(4.2)	17.4(3.4)	15.2(1.6)	19.2(3.0)	23.0(5.3)
	3.2-65.3	3.5-68.3	6.1-24.3	9.2-61.5	7.6-99.8
Cu	30.1(2.9)	33.9(3.2)	33.2(4.4)	35.9(5.4)	28.5(2.4)
	4.7-47.8	12.3-68.6	13.2-66.7	13.7-72.4	16.2-50.2
Zn	**127(14.0)	**165(12.9)	189(19.0)	193(15.3)	185(15.8)
	67.0-258	70.6-253	70.0-297	116-344	106-345
Fe	6429(792)	5371(594)	▲5225(1019)	8519(851)	7796(441)
	2082-9027	697-11206	1384-11326	5146-16369	4795-11562
Mn	598(128)	1067(206)	1258(390)	933(147)	1376(56.5)
	122-1916	182-3386	253-3597	359-2353	227-10117

* Significantly lower than sites 2,3(p=0.01), 4(p=0.001) and 7(p=0.01)
** Significantly lower than at sites 3(p=0.02), 4(p=0.01 and 7(p=0.02)
▲ Significantly lower than at site 4 (p=0.05)

in the water. However, at site 4 the same metals peaked in the alga whilst in the water they declined.

Table 7 gives metal content of *Cladophora* growing adjacent to the Buckhurst Hill drain (RO2), in Chigwell Brook (RO3b) close to where the M11 runoff enters and just below the M11 runoff pipe(RO3a). Individual dates are given since collection was made only on a very occasional basis, growths being few and sporadic.

Copper and cadmium concentrations were generally similar to those recorded in algae at site 7 and nickel was similar for most of the time. Most notable are the data obtained for zinc and lead. The range of lead concentrations in the algae growing in RO3a and RO3b (46-394 µg g^{-1}) were considerably greater than that at the nearby site 7(25.6-150µg g^{-1}). Indeed

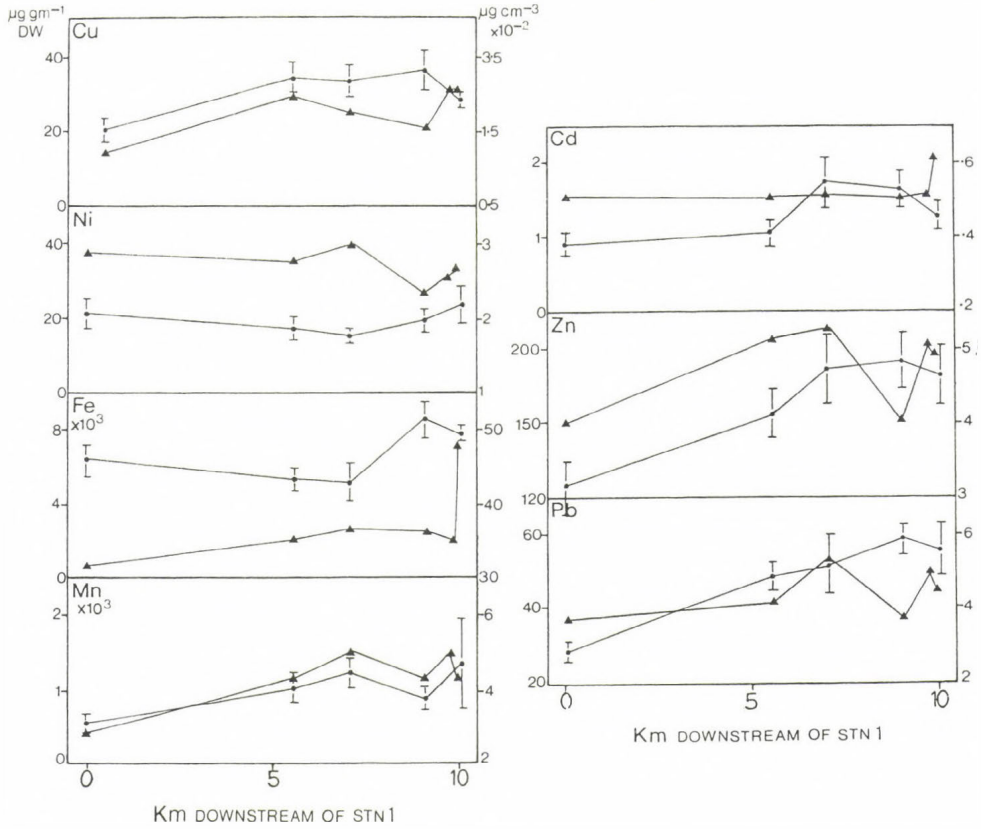

Fig.3. Concentrations of metals in water ▲——▲ and *Cladophora glomerata* ●——● at increasing distances downstream from site 1.

Table 7. Concentrations of metals in *Cladophora glomerata* from inputs on selected dates (µg g^{-1} D.W.)

Input	Date	Pb	Cd	Ni	Cu	Zn	Fe	Mn
RO2	15.8.78	78.0	N/D	14.9	16.4	133	3689	584
	18.4.78	46.0	1.64	13.5	25.8	143	6190	738
	8.5.78	69.0	0.94	6.9	18.3	111	3907	224
	5.6.79	80.8	-	16.2	18.7			
RO3a	12.4.78	108	1.18	17.6	45.7	332		2378
	25.5.78	55.0		17.3	15.5	138		280
	29.5.79	84.3		10.4	17.7			562
RO3b	22.11.77	394	2.09	25.1	37.3	347		3143
	14.12.77	217	1.25	20.4	17.7	298		4641
	8.5.79	218		5.5	66.8	190		285
	29.5.79	386		17.6	45.5	206		891
	5.6.79			7.3	40.4	165		624

levels in *Cladophora* in RO3b were 4 to 5 times higher than those in the alga at any site on corresponding dates in November and December 1977.

Relationship between metals in water and metals in *Cladophora glomerata*

Product-moment correlation was utilised to analyse the relationship between metals in plants and water. Correlation coefficients along with significance levels are given in Table 8.

Table 8. Correlation coefficients for the relationship between metals in *Cladophora glomerata* and metals in water

Metal	Correlation coefficient	Significance
Ni	-0.06	N.S
Pb	+0.09	N.S
Mn	+0.17	N.S
Cu	+0.19	N.S
Cd	+0.26	0.05
Zn	+0.46	0.01

N.S Not significant

Significant correlations were obtained for cadmium(+0.26) and for zinc (+0.46) and scatter diagrams depicting these relationships are illustrated in Figs. 4a and 4b.

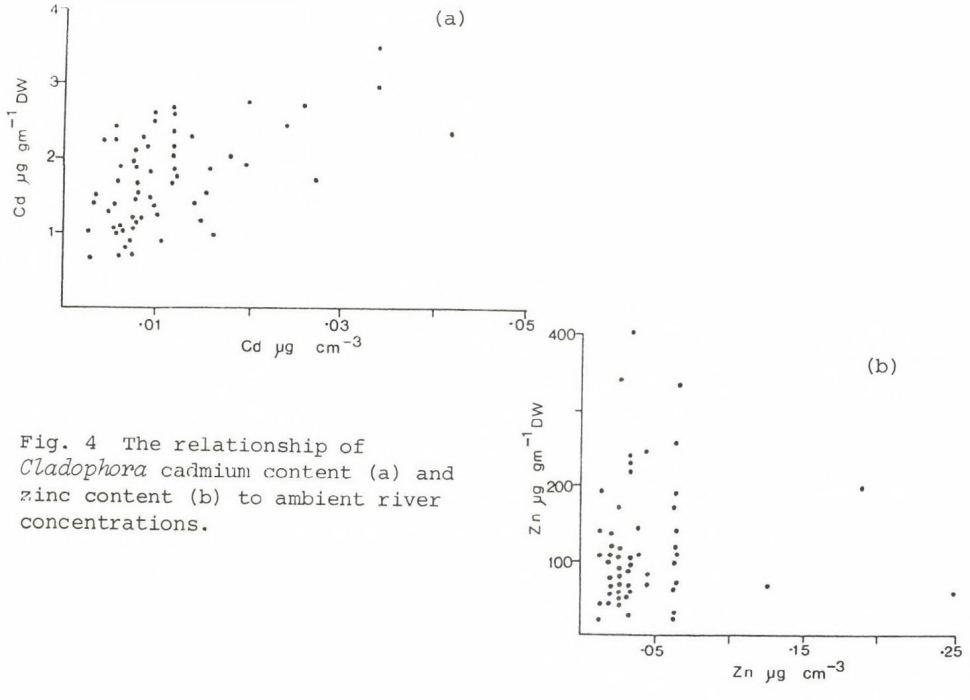

Fig. 4 The relationship of *Cladophora* cadmium content (a) and zinc content (b) to ambient river concentrations.

Concentration factors for metals in *Cladophora glomerata*

In this study the term 'concentration factor' was defined as the ratio of the metal concentration in the alga to that in the water mass. Water metal concentration was taken to mean 'total' as opposed to 'dissolved'. The former it was felt gave a more accurate idea of the total 'pool' of available metals. A summary of concentration factors calculated for each metal in *Cladophora* is given in Table 9. Both mean and ranges of factors are given for each site.

Table 9. Mean concentration factors for metals in *Cladophora glomerata* from sites on the river Roding 1977-79; Ranges are given

	1	2	3	4	7
Pb	1.1×10^{3}	1.5×10^{3}	1.4×10^{3}	1.9×10^{3}	2.1×10^{3}
	2.3×10^{2} - 3.6×10^{3}	5.1×10^{2} - 3.4×10^{3}	4.4×10^{2} - 3.4×10^{3}	5.7×10^{2} - 4.0×10^{3}	5.4×10^{2} - 7.5×10^{3}
Cd	1.7×10^{2}	2.7×10^{2}	3.6×10^{2}	3.6×10^{2}	2.8×10^{2}
	$2.3 \times 10_{2}$ - 4.2×10^{2}	$3.8 \times 10_{2}$ - $.7.6 \times 10^{2}$	$4.4 \times 10_{2}$ - 9.2×10^{2}	$3.2 \times 10_{2}$ - 8.1×10^{2}	$1.3 \times 10_{2}$ - 1.5×10
Ni	1.3×10^{3}	7.3×10^{2}	5.9×10^{2}	6.3×10^{2}	1.3×10^{3}
	2.5×10^{2} - 6.5×10^{3}	1.7×10^{2} - 3.4×10^{3}	1.6×10^{2} - 1.3×10^{3}	2.5×10^{2} - 1.1×10	3.3×10^{2} - 5.0×10^{3}
Cu	1.2×10^{3}	1.7×10^{3}	1.8×10^{3}	2.6×10^{3}	1.6×10^{3}
	4.2×10^{2} - 2.2×10^{3}	2.5×10^{2} - 4.1×10^{3}	6.0×10^{2} - 3.9×10^{3}	2.5×10^{2} - 9.8×10^{3}	5.6×10^{2} - 2.7×10^{3}
Zn	3.9×10^{3}	4.0×10^{3}	3.6×10^{3}	3.8×10^{3}	3.7×10^{3}
	1.1×10^{3} - 8.8×10^{3}	1.2×10^{3} - 9.9×10^{3}	1.1×10^{3} - 8.5×10^{3}	1.2×10^{3} - 8.2×10^{3}	8.8×10^{2} - 7.9×10^{3}
Fe	2.2×10^{4}	1.7×10^{4}	1.5×10^{4}	3.0×10^{4}	2.6×10^{4}
	3.3×10^{3} - 3.9×10^{4}	3.9×10^{3} - 3.6×10^{4}	1.4×10^{3} - 4.1×10^{4}	6.3×10^{3} - 5.7×10^{4}	5.5×10^{3} - 6.0×10^{4}
Mn	2.0×10^{4}	2.5×10^{4}	2.7×10^{4}	3.3×10^{4}	2.6×10^{4}
	7.1×10^{3} - 6.4×10^{4}	2.8×10^{3} - 4.9×10^{4}	4.3×10^{3} - 7.2×10^{4}	1.1×10^{4} - 1.4×10^{4}	4.8×10^{3} - 1.1×10^{5}

Factors were remarkably constant for each metal. This was particularly the case for zinc which varied from a mean of 3.6×10^{3} at site 3 to 4.0×10^{3} at site 2 and displayed a range of factors 1.1×10^{3} to 9.9×10^{3}. Concentration factors were similarly constant for copper with means ranging from 1.2×10^{3} at site 1 to 2.6×10^{3} at site 4. Least consistency was obtained for nickel (5.9×10^{2}-1.3×10^{3}).

DISCUSSION

Mean metal concentrations for water samples taken from sites in the river Roding (1977-1979) reflected largely the degree of metallic input into the system(Fig.2). Metals were low at site 1 (control) although occasional high levels of cadmium at this site could be attributed to the use of superphosphate fertilizers on farmland. The increase in metals at site 2 could be related to a) poor quality sewage; b) industrial estate; c) urban runoff; d)close proximity of M11 motorway. There was evidence from data for inputs into the river (Table 4)that these at times were responsible for elevated metal concentrations at sites 3, 5 and 6.

Fig. 3 illustrates that the overall tendency was for mean metals to increase downstream of site 1 and similarly for *Cladophora* mean metal content. Zinc, copper and lead all increased dramatically at site 2 in the alga. Significantly higher levels of zinc and lead in *Cladophora* in the urban zone highlighted the importance of road runoff, in this case the M11 motorway.

Fig.3 also indicates lack of agreement between metals in water and in algae at site 4. This could be due to the following:- (1) High concentrations of zinc, copper, lead and iron occurred only intermittently at this site and so were not monitored in the water samples. (2) Although metals were lower in the water here, their particular chemical form may have made them more available for uptake by algae. The significantly higher iron content of the alga at site 4 was similarly not reflected in the water chemistry many metals actually declining in the water at this site. A relationship with elevated chlorides has been considered(Stone,1981).

Metal content of *Cladophora* growing in the Chigwell Brook just below where the motorway runoff enters(RO3b) was evidence of intermittently high zinc and lead(Table 7). Maximum concentrations in the algae coincided with high discharges. The comparatively lower concentrations of metals in *Cladophora* growing on the wall beneath the motorway runoff (RO3a) could be explained as follows:- The runoff especially during surges would pass quickly over the algae, resulting in little contact time with the inner filaments whilst in contrast, that growing in the brook(RO3b) would be constantly bathed by water with longer contact time albeit more diluted effluent.

Metals were present in greater concentrations in *Cladophora* than in the water (Tables 3 and 6). and orders of magnitude were also different: Fe Mn Zn Pb Cu Ni Cd *Cladophora* Fe Zn Mn Pb Ni Cu Cd Water Other authors have concluded these discrepancies to be evidence for enhanced or reduced uptake of certain metals (Trollope and Evans, 1976).

With regard to actual metal concentrations recorded in *Cladophora* in the river Roding, concentrations of 82.2-226 $\mu g\ g^{-1}$ and 21.4-41.2 $\mu g\ g^{-1}$ for zinc and lead respectively in the river Wear(Lloyd,1977)compare favourably with the findings of this study (Table 6) although copper (8.08-16.6 $\mu g\ g^{-1}$) and cadmium (1.44-3.13 $\mu g\ g^{-1}$) differed somewhat. In contrast, Trollope and Evans (1976) recorded far higher concentrations of zinc(120-970 $\mu g\ g^{-1}$), lead (60-90 $\mu g\ g^{-1}$), copper (50-60 $\mu g\ g^{-1}$) and nickel (30-100 $\mu g\ g^{-1}$) in *Cladophora* from freshwater sites in the Swansea Valley.

There was found to be a positive significant correlation between zinc in *Cladophora* and zinc in water (+0.46) and between cadmium in the alga and cadmium in the water (+0.26)(Fig. 4a and 4b). Lloyd(1977) and Trollope and

Evans(1976) also recorded significant correlations for zinc in *Cladophora*.

Some concentration factors were found to be remarkably constant with time, indicating the existence of a proportional relationship between metals in water and algal tissue. Zinc displayed most constancy (Table 9) with means ranging from $3.6x10^3$ to $4.0x10^3$ at all sites. This stability for zinc has been noted by other workers (Trollope and Evans,1976) as has the lack of consistency for nickel.

Other research on metal concentration factors in *Cladophora* shows close agreement with the findings of this survey. This was particularly the case for copper where the Roding values ($1.2x10^3$-$2.6x10^3$) compared favourably eg. Funk et al. (1973) $2.5x10^3$, Taft and Kischler(1973) $1.0x10^3$, Keeney et al.(1976) $1.9x10^3$-$2.2x10^3$, Trollope and Evans (1976)$1.8x10^3$-$3.5x10^3$, Lloyd (1977) $1.9x10^3$. Similarly concentration factors obtained for lead in *Cladophora* in this study ($1.1x10^3$-$2.1x10^3$) fell within the range obtained by other workers($9.0x10^2$-$1.6x10^4$).

This survey demonstrated similar trends for water metals and algal metals particularly at the lower concentrations and total agreement for manganese. Some of the findings at site 4 proved an exception to this. This, along with the fact that *Cladophora* indicated significant differences in metals that the water did not suggests that the alga is possibly of more value as a monitor of certain heavy metals than the water itself. Thus reasons for using *Cladophora glomerata* as a biological monitor of heavy metals in an urban river are outlined below:-

(1) High concentrations of metals, most notably zinc and lead are accumulated without death occurring.

(2) The alga is attached to the substratum and so it is representative of the collection area.

(3) It is abundant for most of the year.

(4) Its reasonable size provides adequate tissue for analysis.

(5) It is easy to sample.

(6) It exhibits high concentration factors for metals, allowing direct analysis.

(7) Concentration factors for some metals such as zinc and copper are remarkably stable.

(8) A simple correlation or proportional relationship exists between zinc and cadmium content of the organism and the average metal concentration of the surrounding water.

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DISCUSSION

THEEDE, H: Do you know, what portion of heavy metals is really incorporated by the algae and what is only adsorbed? Is there a great variability of adsorption caused by epiphytes?

McHARDY, B: /1/ This question was not looked at in this particular study but in later laboratory investigations uptake of zinc and lead by the alga were certainly found to be an irreversible process.

/2/ Again this is outside the scope of this particular study. The epiphytes may not play a very important role in adsorption but I believe that this has not been satisfactorily investigated by anybody for Cladophora. Certainly there were no obvious seasonal trends in the results as you would expect if epiphytic growths were playing a major role /epiphytic growths make up a higher percentage of total Cladophora biomass in summer old growths than winter and autumn new growths/.

BRIX, H: I understand, that you collected all the tissue of the Cladophora, and you told us, that the plants in some instances were more than 3 meter in length. Have you investigated if the concentrations of heavy metals in the old plant tissue differred from the concentrations in the young tissue?

McHARDY, B: Yes, this was looked at in brief and it was found that unlike with higher plants there was no real difference in concentrations. Collecting Cladophora of this length was generally avoided since it tended to be highly silted and contaminated with foreign matter. Since growth was very rapid these lengths were quickly attained and soon became detached anyway. In actually collecting plant tissue generally the last 10 cm of filament was normally collected and bottom silted portions avoided.

WACHS, B: In one summary point you've said that the concentration factors are stable and that could be seen positive for bioindicators. In river ecosystems generally the factors do change due to the metal concentrations in water.

McHARDY, B: Yes, but whilst metal concentrations in the water change, so does metal content of the alga. Thus for metals such as copper and zinc where there seems to be a proportional relationship between water metals and alga metals, the range of concentration factors produced is very narrow

whilst for nickel it is far greater. So perhaps it would be better to say "relatively stable" or "relatively constant".

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HEAVY METAL ACCUMULATION AND PHYSIOLOGICAL EFFECTS ON AUSTRIAN MACROPHYTES

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INTRODUCTION AND METHODS

This paper shall be concerned with studies made in the course of a thesis in 1981. The experiments were carried out by Dr. Ledl, whereas Dr. Horak was in charge of the determination of the heavy metal levels. The author was responsible for the assignment of the topic, the supervision of the scientific project and the analyses of the organic substances; the latter were supported by the "Fonds zur Förderung der wissenschaftlichen Forschung", project no. 3042.

The material for these studies was mainly taken from rivers in Lower Austria. After sampling, the apical 20-30 cm tips of the material were cleaned from adhering detritus and sand, rinsed in distilled water and brought to the laboratory, where they were transplanted into 60 l plastic containers filled with a 5 cm thick layer of sand. Pure tap water was used as a culture medium. The plants were cultivated under flowing water conditions; the water was circulated by a pump, the backflow of the solution from the growing container was achieved by two tubes, the large inner diameter of which guaranteed the backflow even in the case of bubbles.

For the purpose of exposing the plants, which had acclimated for at least three weeks, to the individual heavy metals they were transplanted into 2o l plastic containers and each time incubated with one of the heavy metals to be studied. During the long-term experiments, which extended over a period of fourteen days, the solution was changed every second day, thus ensuring as constant a concentration as possible throughout the course of experiments. The uptake of heavy metals occurring on the entire surface of plants has already been the subject of

studies made by other researchers [1] .

The following heavy metals were used in the experimental setup: copper, cadmium and nickle. Cu was selected on account of its high phytotoxicity, Cd on account of its frequent occurrence in anthropogenically influenced systems and its animal toxicity and Ni because it is known that only relatively small quantities of this element are absorbed by plants. The following species were selected for the experiments: Myriophyllum spicatum, because it is fairly common in flowing and still waters in Lower Austria, Potamogeton coloratus as a plant typical of non-polluted waters and Potamogeton pectinatus as a plant characteristic of highly eutrophic waters, tolerating even waters strongly polluted by heavy metals.

The heavy metals were analyzed by means of atomic absorption spectrometry, the organic substances by gaschromatography (sugar, organic anions) or by enzymatic assay for starch and by means of an automatic amino acids analyzer (AAA) for the amino acids.

RESULTS AND DISCUSSION

At first we wanted to ascertain whether the uptake pattern of heavy metals is reproducible within a certain period of time: for this purpose short-term experiments, which lasted for four days, were set up. Of the two concentrations used only the results of the 0,1 ppm-series are reported. This concentration is more similar to natural conditions and, moreover, it is likely to result in higher uptake and accumulation rates [2].

If we compare the best and the worst results of our experiments, the values detected in the same experimental setup - consisting of four identical replicates - but also at different dates within the vegetation period (May - November) were almost invariably in the range of the 95% confidence limits, which is about twice the standard deviation. This is not self-evident for we know from the literature [3] that the heavy metal level may be subject to seasonal changes. But this does apparently not hold true for the uptake within relatively short periods of time as , for instance, the two weeks observed in our experiments. As we can see, it had obviously only little influence on the uptake whether the material had been harvested in May or in October. If we look at the accumulation factors for heavy metals in the test plants, we realize that the values range from 10^3 to 10^4 . Thus, they closely resemble the accumulation values

detected in plant material stemming from polluted sites on Lower Austrian rivers. The same applies to the values recorded by Wachs [4] on the bioaccumulation of Cu, Cd and some other heavy metals in macrophytes. Considering these field values in detail, we see from the samples investigated that here, too, Ni was the element with the lowest accumulation, the factors ranging from 400 to 7000 in Austrian phanerogams and amounting to 1200 in bryophytes (Fontinalis). Our group but also Wachs found Cu - in some cases showing up to 150 000-fold concentrations - to be the element with the highest accumulation among the microelements (Fig. 1-3).

There was, however, a certain difference between samples taken from areas with silicate substrata and those collected in the carbonate region. Nevertheless, almost equally high levels of heavy metal accumulation have to be expected because the lower reaches of Lower Austrian rivers in the carbonate region are usually fairly industrialized.

The heavy metals studied in plants sampled from natural habitats revealed a definite order as far as their enrichment was concerned. In about 20 different species of macrophytes the following metals were enriched in decreasing order: Mn, Fe/Zn, Cu, Cd and Ni. As regards the order of Cu, Cd and Ni, the results of the field studies reflect exactly those of the experimental studies. For the plants sampled in the field we found the following accumulation index: the highest accumlation for the largest number of heavy metals was observed in Fontinalis. In declining order follow Eleodea canadensis, Ranunculus trichophyllus, Ranunculus fluitans, Myriophyllum verticillatum, Potamogeton crispus and Lemna trisulca. Besides the well-known fact that it is the bryophytes which have a very high capacity of heavy metal accumulation we may infer from this order that very frequent plants of our rivers, i.e. Elodea canadensis and Ranunculus trichophyllus, but also Myriophyllum species show a fairly high capacity of accumulation. This is above all important since phanerogams have been only seldom studied recently [5] and preference was almost exclusively given to certain bryophytes [6,7]. This approach is, however, not necessarily justified since bryophytes in general cannot be regarded as a group occurring more frequent than phanerogams and their capacity of accumulation is not always significantly higher than that of the latter.

Returning to our experimental setup, we realize that our results resemble closely those detected in plants which were sampled from natural

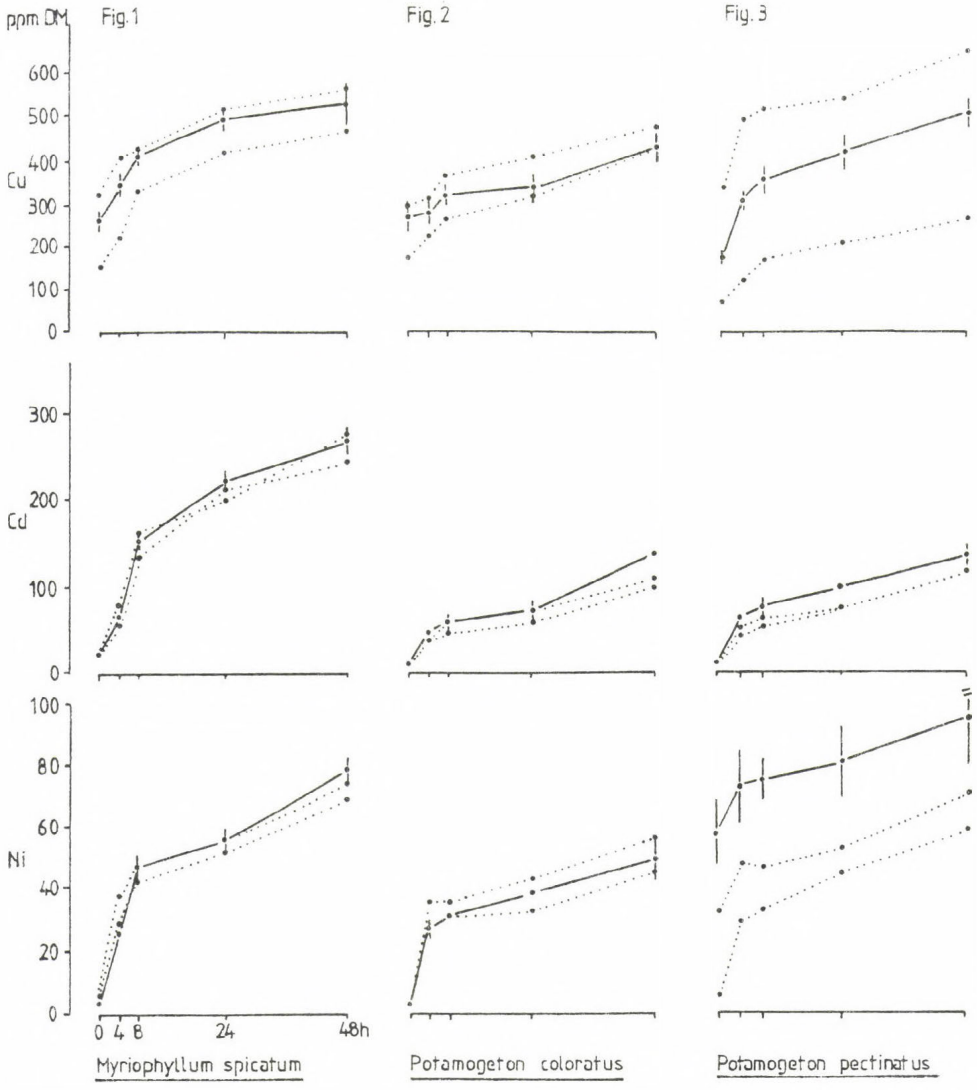

Fig. 1. Myriophyllum spicatum: accumulation of Cu, Cd and Ni. Solid line = four identical replicates, mean +/- S. D. Dotted line = successive tests later in the year.

Fig. 2. Potamogeton coloratus: for description see Fig. 1

Fig. 3. Potamogeton pectinatus: for description see Fig. 1

habitats.

For about ten years our team has been analyzing the organic substances of aquatic plants as well. These include above all starch and soluble carbohydrates as well as organic anions and the amino acids. It was shown among other things that, for instance, the level of starch or that of different sugars or potassium may be an indicator for the eutrophication effects on aquatic plants. It was therefore obvious that the heavy metal accumulation, which occurred at so regular periods of time, was sufficient reason to analyze these substances, too, in order to find out whether they are subject to similar, regular changes. Since so far no more detailed studies on the actual dependence of certain metabolic reactions due to the heavy metal uptake have been made by us, I should like to report just briefly on the results.

If the plants did not die within the exposure time of two weeks - as, for instance, did Myriophyllum spicatum under the effect of Cu - the starch level invariably increased irrespective of the heavy metal added or the species analyzed. This is an unexpected result in so far as we may infer from studies made by Raabe, Schuster and Kohler [8] that Cu inhibits the net photosynthesis significantly and may even affect the activity of different dehydrogenases. This did obviously not happen with the two Potamogeton species. It is also unclear whether the results were brought about by increased starch formation or, for example, by inhibited decomposition (Fig. 4).

The soluble carbohydrates - similar to starch - in almost all cases showed a higher concentration after a fortnight than at the start of the experiment. This is possibly due to excessive transport activities and to the activation of resistance mechanisms; but this phenomenon, too, has not been verified by direct proof (Fig.5).

In accordance with the relevant literature the results for the organic anions might be somewhat easier to interprete. Just as in senescent plants or in plants damaged by environmental pollution the organic level is increased. A further reason for this phenomenon may lie in the fact that organic anions might be also regarded as sites where some of the heavy metals are bound and thus detoxified; we know, for example, Fe-citrate compounds, but also Ni-malonate and Ni-malate complexes (Fig.6).

In contrast, the amino acids usually decline towards the end of the fortnight. For this phenomenon, too, no explanation proven by experiment

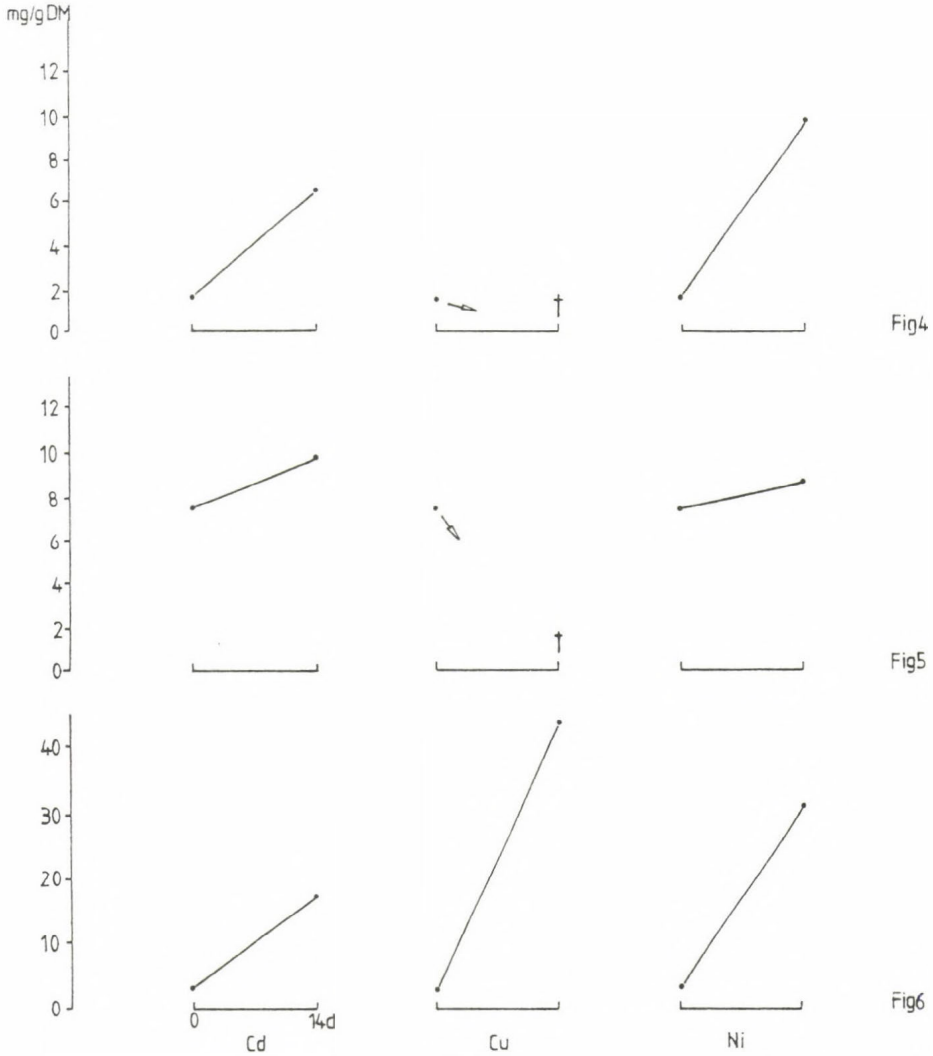

Fig.4. Myriophyllum spicatum: starch, concentration of heavy metal 0.1 ppm .

Fig.5. Myriophyllum spicatum: soluble carbohydrates, for concentration see Fig.4 .

Fig.6. Potamogeton coloratus: organic anions, for concentration see Fig.4.

can be provided at the moment. Whether a generalization of this decrease is possible, will have to be verified by further studies of plant material. We might, of course, now speculate on the most diverse effects on all kinds of enzymatic systems; however, this shall not be the main issue of my speech. Our interest centers rather on the issue of a bioindication.

a) As for the organic substances, the increase in the carbohydrate and organic level must certainly be referred to as indicative, yet we would have to record exactly the level pattern within shorter periods of time and test by a series of experiments whether it is reproducible. If we are not only interested in the purely physiological aspect, but also aim at its practical application, the analysis of organic substances may prove to be somewhat too slow and, moreover, the increase in the carbohydrate and organic anion level to be not typical of heavy metal pollution. In addition, there is the difficulty that, for instance, old leaves have generally a higher heavy metal level than young ones [9] .

b) If we want to put a simple tool for the biomonitoring of heavy metals to practical use, we should - in view of the fairly simplified analysis today - not only pay special attention to the high accumulation capacity of the biomonitor, but also consider its easy availibility, that is to say, its wide distribution within a certain area as an important criterion. This means that not just bryophytes [10] are suitable as monitor organisms - as is the case in England - besides Fontinalis also Elodea canadensis and Myriophyllum species may be used. Just as important in my opinion is , however, to discard the widely held view that the use of macrophytes as passive monitors is the most convenient method. If we know exactly the uptake behaviour of aquatic plants, it is much more convenient to use these plants as active monitors [11] within standard periods of exposure. Thus, we avoid the difficulty encountered frequently that in particular at sites with intermittent discharge of pollutants neither the right plant nor any vegetation at all may be observed.

In the near future our team intends to work out the standard conditions for such active monitoring and to provide for the appropriate devices required for the planting and recollecting of the monitor plants.

In conclusion, it may be said that one of the most important findings revealed in this study is the fact that the changes in the organic substances caused by heavy metal pollution proved to be reproducible. For this reason we may also rely on the changes in the level of organic sub-

stances in macrophytes as an indicative system of heavy metal pollution.

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DISCUSSION

SALÁNKI, J: It was very consequent that Cu, Cd and Ni were taken up to different degrees by the three plants. Do you have any data on the metabolic activity of them what could be one of the reasons of these differences?

For the explanation of the differences in Cu, Cd and Ni content one could assume that transportation or release of these metals is different from the apical part you are investigating. What is your comment to that?

JANAUER, G: As for the metabolic activity I have no information so far. The differences of the contents of the individual metals cannot be claimed for by transport processes, since the apical parts investigated were collected as a whole for metal analysis, including the few small roots that had developed while acclimatizing the material in the growth containers. Moreover, as shown by other authors at the symposium, other water plants, no matter if from Germany, USA or Hungary, show the same effect, namely individual accumulation factors for each individual heavy metal.

THEEDE, H: I would like to mention that Dr. Dickmann from our institute found a dependence of Cd-uptake on light conditions in the sea grass Zostera marina. Therefore I wonder that in Potamogeton species there were only so small seasonal differences in the uptake rates of heavy metals.

JANAUER, G: During the period of collection of plant material in the field and during the experiments, both between May and September, no low-light conditions depending on elevation of sun, are possible. In addition to that plants grow up to the surface in the course of the year, so they may experience at least as good light conditions in September as in May.

LORCH, D: In Chlamydomonas under the influence of lead starch and lipid metabolism were influenced. Was lipid analysis carried out?

JANAUER, G: Being still tied up with extending our methods of carbohydrate analysis and related substances - which is fairly highly advanced in an international comparison - we have not yet turned into lipid analysis, though the apparative outfit /gas chromatography/ is present; the instrument is permanently used for sugar analysis and determination of organic anions. Also the lipid level is low in submerged macrophytes as compared to some terrestrial plants and at present there is no possibility to introduce these new methods to our working-group.

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DISTRIBUTION OF HEAVY METALS IN AQUATIC MACROPHYTES FROM OKEFENOKEE SWAMP

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ABSTRACT

Elemental concentrations of eight aquatic macrophytes: white water lily (Nymphaea odorata Ait.), yellow water lily (Nuphar advena Ait.), pickerel weed (Pontederia cordata L.), yellow eyed grass (Xyris smalliana Nash.) beakrush [Rhynchospora inundata (Oakes) Fern.], redroot [Lachnanthes caroliniana (Lam.) Dandy], sphagnum (Sphagnum cuspidatum var. serrulata Schlieph.), and bladderwort (Utricularia sp.) were measured in Okefenokee Swamp during October 1977. Iron, aluminum, copper, zinc, silicon, cobalt, chromium, nickel, lead, cadmium, and strontium were analyzed in plant parts and in various Okefenokee locations. Mercury was also analyzed for in Utricularia. In general, concentrations were lower than in the same species found outside Okefenokee Swamp, which reflects its unpolluted and ombrotrophic state. Differences in plant part explained more variation in elements than location. Nuphar stems, Utricularia, Lachnanthes leaves, and Sphagnum had highest concentrations of most elements.

INTRODUCTION

Previous studies have examined elemental concentrations of Okefenokee macrophytes (Casagrande and Erchull, 1976; Schlesinger, 1978; Bosserman, 1979; 1981). These studies concluded that major elements were accumulated by upper plant parts while heavy metals were accumulated by roots and peat. This research examined the distributions of various elements (Fe, Al, Cu, Zn, Si, Co, Cr, Ni, Pb, Cd, Sr, and Hg) in eight common aquatic macrophytes in Okefenokee Swamp (Nymphaea odorata, Pontederia cordata, Nuphar advena, Xyris smalliana, Rhynchospora inundata, Lachnanthes caroliniana, Sphagnum sp., and Utricularia sp.). Certain of these elements are recognized as heavy metal pollutants in disturbed environments. Okefenokee Swamp is not impacted by any major sources of heavy metal pollution, although it may be impacted by acid rain. There were also several bird rookeries in the swamp which provide sources of natural eutrophication.

Sampling was done in October 1977, after a drought of several months. Variations among plant parts, among swamp locations, and among plant species were assessed to gain a preliminary understanding of the elemental distribution in Okefenokee marshes. Okefenokee macrophytes were also compared with macrophytes which have been studied in other regions. Results complement previously documented analyses and discussions of elements in Okefenokee macrophytes (Bosserman 1979, 1981).

MATERIALS AND METHODS

Study Area

Okefenokee Swamp occurs in southeastern Georgia within a shallow basin which contains up to 14 feet of peat (Cohen 1974). Element inputs occur largely through precipitation and runoff from uplands in the northwestern part of the watershed. These sources contain few minerals and result in low dissolved element concentrations (Rykiel 1977, Bosserman 1979). There are no known sources of ground water input.

Water level is usually less than 0.5 m deep and varies through the year depending on evapotranspiration, precipitation, and river outflow (Rykiel 1977). Frequent droughts occur in Okefenokee marshes which cause plant death, concentration of available elements, rapid decomposition of organic matter, and fires. Although most Okefenokee droughts occur in fall and winter, a drought occurred in summer 1977 before the October sampling period. At the time of sampling, water level was nearly equal to the annual average.

Three sites were chosen in each of seven marshes within Okefenokee Swamp: Grand Prairie, Chesser Prairie, Chase Prairie, Billy's Lake, Floyd's Island Prairie, and Mack's Island Rookery. The Chesser and Grand Prairies sites were shallow marshes that were exposed during the 1977 drought, while the Chase, Durdin, and Floyd's Island sites were deeper marshes that did not

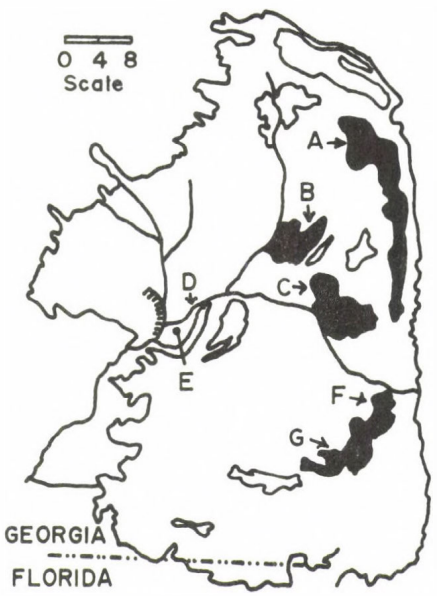

Figure 1. Map of Okefenokee Swamp indicating the location of marshes where sampling occurred. A = Durdin Prairie, B = Floyd's Island Prairie, C = Chase Prairie, D = Billy's Lake, E = Mack's Island Rookery, F = Chesser Prairie, and G = Grand Prairie.

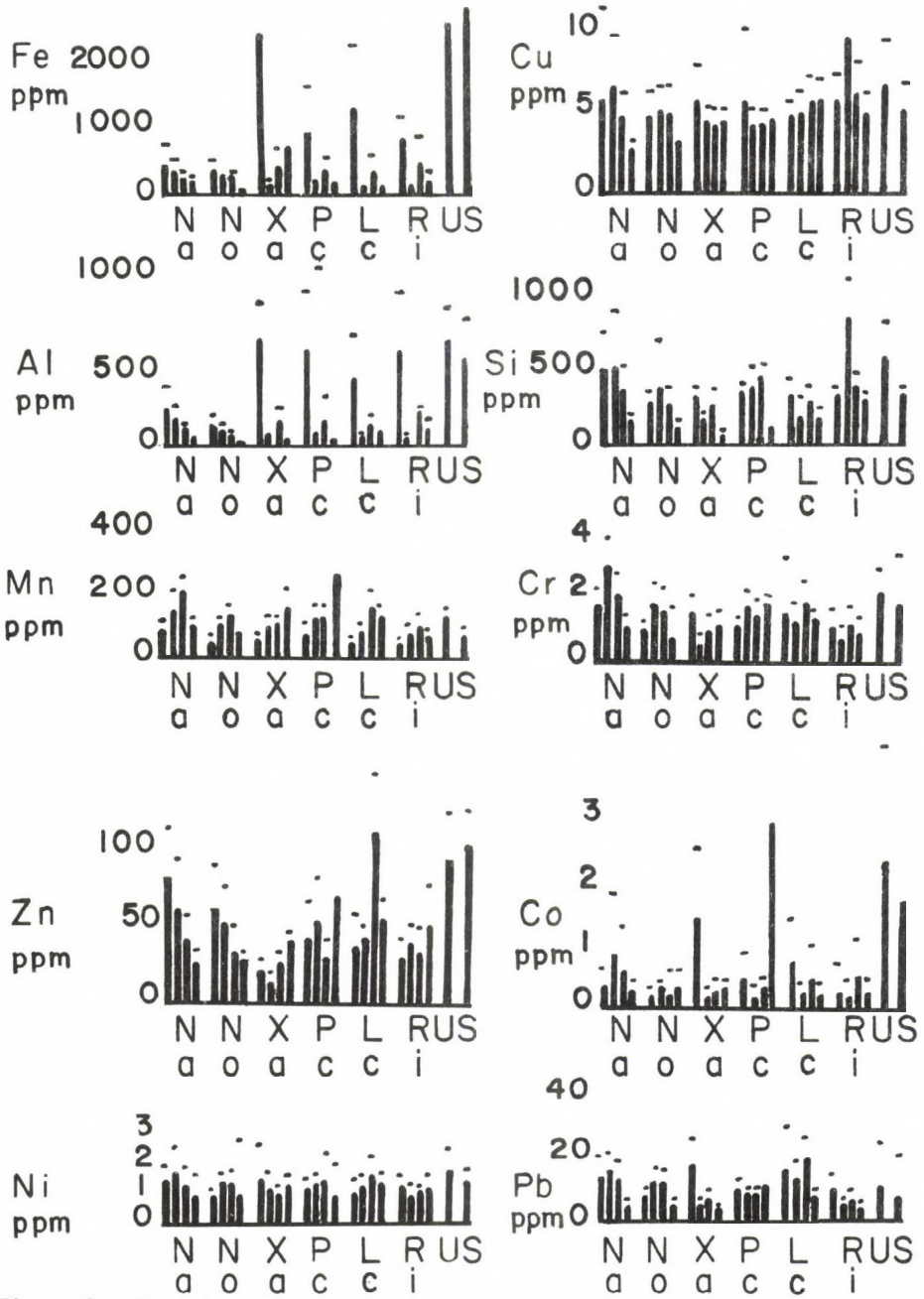

Figure 2. Mean concentrations and standard deviations of various metals in Okefenokee macrophytes: Nuphar advena (N.a.), Nymphaea odorata (N.o), Xyris ambigua (X.a.), Pontederia cordata (P.c.), Lachnanthes caroliniana (L.c.), Rhynchospora inundata (R.i.), Utricularia sp. (U.) and Sphagnum (S.). For each plant the bars from left to right represent concentrations in roots (or rhizomes), stems (or petioles), leaves, and flowers.

dry up completely during the drought. Billy's Lake plants were sampled from small marsh areas adjacent to a shallow lake. Mack's Island Rookery is a naturally eutrophied area which receives nutrient input from roosting and nesting birds. Concentrations of dissolved P, N, K, and Na were elevated in rookery water. Likewise, plants within the Mack's Island Rookery have elevated concentrations of P, N, K, and Na (Bosserman 1981).

Methods

Eight macrophyte species were sampled in each marsh when present: Nymphaea odorata, Nuphar advena, Pontederia cordata, Xyris smalliana, Lachnanthes caroliniana, Rhynchospora inundata, Utricularia sp., and Sphagnum sp. Each sample included three to five plants, except for Sphagnum and Utricularia samples which included numerous plants. Utricularia was represented by three species (U. inflata Walt., U. purpurea Walt., and U. juncea Vahl.); these species were entangled and were difficult to separate. Individual Sphagnum plants were not identified; however, S. cuspidatum var. serrulata has been identified as the most common Sphagnum species in Okefenokee Swamp (Bosserman 1979). At sampling time, Nuphar, Nymphaea, Utricularia juncea, and Pontederia were flowering; Xyris, Lachnanthes, and Rhynchospora were past flowering, although they retained senescent flowers and seeds. Utricularia was the only plant found in all sites.

After sampling, macrophytes were separated into roots, stems, leaves, and flowers; dried in a forced air drying oven at 60° C; and ground in a Wiley mill. One gram of sample was ashed at 500° C in a muffle furnace; dissolved in a 20% nitric acid solution; and analyzed on a Jarrell Ash plasma emission spectrometer. Mercury analysis was done with a Coleman mercury analyzer (Hatch and Ott, 1976). Statistical analyses were done using the General Linear Modelling and Means Procedures of Statistical Analysis Procedures (SAS) (Barr et al., 1976). The Duncan multiple range test was used to test differences among means at a 0.05 level.

RESULTS AND DISCUSSION

All values in the following results are means in parts per million (g/g). Mean values and standard deviations are plotted in Figure 2.

Iron. Mean Fe concentrations (Fig. 2) were highest in Utricularia (2700) Sphagnum (2900), and the roots of Xyris (2500), Ponterderia (900), Lachnanthes (1300), and Rhynchospora (800). The submersed parts of all plants had higher concentrations of Fe than other parts. Flowers of all plants, except Xyris, had low concentrations of Fe. Iron concentrations were lower for Okefenokee Nymphaea and Nuphar than for the same plants studied in other parts of the U.S. (Table 1), while the concentrations for Okefenokee Pontederia and Utricularia were intermediate.

Aluminum. Aluminum concentrations (Fig. 2) showed the same trends as Fe. Mean Al concentrations were highest in Utricularia (670), Sphagnum (570), and roots of Xyris (700), Pontederia (370), Lachnanthes (450) and Rhynchospora (630). Flowers of all plants, except Lachnanthes and Rhynchospora, had lowest concentrations of Al. The concentration of Al in Okefenokee Nuphar and Nymphaea was lower than in the same plants in Connecticutt (Table 1).

Copper. Mean Cu concentrations of Okefenokee aquatic plants are shown in

Figure 2. No significant differences existed among parts of Nuphar and Nymphaea, although Cu tended to be lower in the flowers. Highest concentrations occurred in Rhynchospora stems (9.6), Nuphar stems (6.7) and Utricularia (6.7). Roots of Xyris and Pontederia had higher concentrations than upper plant parts; contrarily, leaves and flowers of Lachnanthes had higher Cu than stems and roots. Copper concentrations in Okefenokee plants were lower than in the same species which have been studied elsewhere (Table 1).

Zinc. Highest concentrations of Zn (Fig. 2) occurred in Lachnanthes leaves (110), Sphagnum (100) and Utricularia (92.0). Nuphar and Nymphaea roots contained significantly higher Zn than upper plant parts, while flowers had lowest Zn concentrations. In contrast, flowers of Xyris, Pontederia, and Rhynchospora had highest concentrations. Okefenokee plants had lower Zn concentrations than Connecticut plants but had Zn concentrations similar to New Jersey and South Carolina plants (Table 1).

Silicon. Highest Si concentrations (Fig. 2) occurred in Rhynchospora stems (820), Utricularia (560), and Nuphar stems (510). Lowest Si concentrations occurred in flowers of all plants. Nymphaea and Nuphar stems had highest Si concentrations. Silicon concentrations of Okefenokee plants was invariably lower than concentrations of Connecticut plants (Table 1).

Cobalt. Highest Co concentrations (Table 2) occurred in Pontederia flowers (3.02), Utricularia (2.43), Sphagnum (2.00), and roots of Xyris (1.50). Pontederia flowers often had high concentrations of elements (Cowgill 1974, Hutchinson 1975, Bosserman 1981). Concentrations in parts of both Nuphar and Nymphaea were not significantly different. Roots of Xyris and Lachnanthes had highest Co concentrations. In general, Co in Okefenokee plants was higher than Co in Connecticut plants (Table 1).

Chromium. Highest Cr values (Fig. 2) occurred in Nuphar stems (2.92), and leaves (1.96), Nymphaea stems (1.73), Utricularia (2.18) and Lachnanthes leaves (1.89). Nuphar and Nymphaea Cr was highest in stems and leaves and is lowest in flowers. The highest Cr concentrations of Xyris occurred in roots while lowest occurred in stems.

Nickel. Highest Ni concentrations (Fig. 2) occurred in Nuphar stems (1.75), Lachnanthes leaves (1.57) and Utricularia (1.52). Flowers of Nuphar, Nymphaea, and Pontederia had low Ni concentrations relative to leaves and stems. Okefenokee plants had lower Ni concentrations than Connecticut plants (Table 3).

Lead. Highest Pb concentrations of Okefenokee plants (Fig. 2) occurred in Lachnanthes leaves (20.9) and roots (16.9), Xyris roots (19.1), Utricularia (16.9) and Nuphar stems (16.7). Except for Pontederia, flowers of aquatic macrophytes tended to have lower Pb than other parts.

Cadmium. Highest Cd concentrations occurred in Nuphar stems (1.42), Lachnanthes leaves (1.16) and Utricularia (1.15). Flowers of Nymphaea, Nuphar, Xyris, and Rhynchospora had lowest Cd concentrations. Like other elements, Cd in Pontederia flowers was higher than other parts.

Strontium. Highest Sr occurred in Lachnanthes leaves (102.0),

Table 1. Ranges of element concentrations (μg/g) in macrophytes from Okefenokee Swamp and from other aquatic ecosystems (Cowgill* 1973a, 1973b, 1974, Boyd** 1978, Riemer and Toth 1973 *** Adams**** et al. 1973).

Element	Location	Nuphar advena	Nymphaea odorata	Pontederia cordata	Utricularia sp.
Fe	Connecticut*				
	Okefenokee	130-420	300-2100	110-920	1100-7030
Al	Connecticut	230-510	180-1730		
	Okefenokee		90-140	70-630	390-870
Cu	Connecticut	51.6	46.1	47.5	
	South Carolina**	35.0	36.0	60.0	
	New Jersey***	10.2	8.6	16.3	4.01
	Pennsylvania****	23.0			
	Okefenokee	2.7-6.7	3.0-4.9	4.0-5.6	3.8-13.9
Zn	Connecticut	129.3	122.9	126.1	
	South Carolina	50.0	32.0	67.0	10.8
	New Jersey	32.2	34.5	26.5	15.7
	Pennsylvania	65.6			
	Okefenokee	26.8-60.9	30.5-52.8	30.3-71.1	56-149
Si	Connecticut	760-5630	1830-10640	1800-5680	
	Okefenokee	123- 518	89- 374	91- 425	380-820
Co	Connecticut	0.06-0.13	0.70-1.0	0.08-3.2	
	Okefenokee	0.31-0.95	0.25-0.35	0.22-3.02	0.99-6.3
Ni	Connecticut	2.8-3.5	2.9-3.8	2.9-4.0	
	Okefenokee	0.93-1.76	0.91-1.32		0.93-3.0
Sr	Connecticut	27.6-133.9	13.4-23.8		
	Okefenokee	20.9-70.5	9.5-30.7	29.6-65.0	
Hg	Connecticut			33	
	Okefenokee				55.9-105

Nuphar stems (70.0) and leaves (71.0). Strontium was lowest in flowers of Nuphar, Nymphaea, and Rhynchospora. Strontium in Okefenokee plants tended to be similar to Sr levels found in Connecticut plants (Table 1).

Mercury. Mercury concentrations in Utricularia varied from 55.8 -105.0 ppm with a mean of 71.2 ppm and a standard deviation of 15.95. These values are higher than in Pontederia which was measured in Connecticutt (Table 1).

Part Versus Location

The amount of element variation accounted for by the parts and the location of aquatic plants is shown in Table 4. In most cases the differences among parts explain more variation than differences among locations. Location was responsible for significant variation in Utricularia Fe (82%), Cu (84%), Co (75%), Cr (71%), and Pb (61%); Nuphar Zn (29%), and Sr (29%); Nymphaea Fe (45%) and Al (35%); Pontederia Cu (19%), Zn (7%), Co (17%), and Ni (15%); Lachnanthes Al (10%) Cu (18%), Zn (28%), and Si (19%), and Rhynchospora Al (10%), Si (11%), Co (32%), and Ni (34%). Location explains more variation than part in Nymphaea Zn and Si, and Cu, Lachnanthes Cu, and Rhynchospora Co and Ni.

Concentrations of certain elements in Utricularia were higher in the rookery than in other Okefenokee locations. These elements included P (3,400

Table 2. Percent variation explained by plant part and location.

Element	Utricularia sp.	Sphagnum sp.	Nuphar advena		Nymphaea odorata		Xyris smalliana	
	Site	Site	Part	Site	Part	Site	Part	Site
Fe	82*	17	19	9	22*	45*	56*	8*
Al	48*	42	32*	11	29*	35*	84*	1
Cu	84**	19	7	10	18	19	25**	19*
Zn	28	23	32****	29***	36***	36***	73****	5
Si	34	24	11*	21	16*	34***	68****	3
Co	75**	18	16	0	68****	10**	48****	8
Cr	71**	20	3	32*	31***	19**	44***	11
Ni	48	13	5	12	44***	8	27**	1
Pb	61*	11	10	22	56****	5	71****	14
Cd	59	15	1	11	7	17	34**	6
Sr	59	47	5	29*	54****	12*	42****	20***

Element	Pontederia cordata		Lachnanthes caroliniana		Rhynchospora inundata	
	Part	Site	Part	Site	Part	Site
Fe			48*	9	62*	10
Al			56*	10	65*	10*
Cu	13*	19**	15***	18****	20	13
Zn	59****	7**	28****	28****	21*	10
Si	27***	2	45***	19*	57***	11***
Co	25****	17***	76****	9	12	32**
Cr	6	13	41**	11	13	18
Ni	24****	15**	20	8	4	34*
Pb	23***	6	36**	9	39**	11
Cd	37****	9	16*	29	9	7
Sr	66****	4	59***	8	26**	11

ppm) K (17,200 ppm) and Na (13,000 ppm) (Bosserman, 1981). In contrast, some of the heavy metals were lowest in the rookery Utricularia: Fe (1,100), Cu (3.8), Al (409), Co (0.99), Cr (1.48) and Ni (1.14).

CONCLUSIONS

The conclusions of this study can be summarized by several statements. Heavy metal concentrations in Okefenokee aquatic macrophytes tend to be lower than in the same macrophytes which have been studied elsewhere in the United States, except for Utricularia. Okefenokee Utricularia had higher concentrations of several heavy metals, including Cu, Zn, and Hg, than Utricularia which had been studied by other U.S. researchers.

In Okefenokee Swamp, Utricularia, Sphagnum and the roots of several aquatic plants have high concentrations of Al, Fe, Zn, Pb, and Cd. This result is consistent with the finding of earlier Okefenokee researchers that peat and plant roots had high concentrations of heavy metals. Nuphar stems and Lachnanthes leaves were also notable for their high concentrations of elements.

Flowers have the lowest concentration of many elements studied, except for Pontederia flowers which have high concentrations of many elements. The reason for the high concentrations in Pontederia flowers is unknown but this result reinforces the findings of other studies (Hutchinson, 1975).

Location explained much elemental variation in Okefenokee aquatic macrophytes, although plant part explained most variation. In a rookery area where N, P, Na, K, and Ca were present in high dissolved concentrations, N, P, Na, K and Ca were in higher concentration in Utricularia than in other Okefenokee locations, while Fe, Al, Cu, Co, Cr, and Ni were present in lower concentrations.

Aquatic macrophyte marshes, which cover 15-20% of Okefenokee Swamp (Bosserman, 1979), include much of the floral and faunal diversity of Okefenokee Swamp. Knowing the elemental composition of Okefenokee aquatic macrophytes is important to understanding elemental cycling within this wetland ecosystem. Many of these elements are major constituents of anthropogenic pollution; however, Okefenokee Swamp is a relatively undisturbed environment which has suffered little impact from heavy metal sources. Like other studies of Okefenokee Swamp (Bosserman and Hagner, 1981), this study demonstrates that this wetland ecosystem has not been greatly affected by pollution. There are few sources of pollution in the Okefenokee environment. Such research helps to provide a baseline for comparison with disturbed ecosystems and adds to the basic knowledge of wetland environments.

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DISCUSSION

SHIBER, J.G: You have mentioned that totally submerged and free-floating plants are "ideal indicators" of heavy metals. Shouldn't this apply to a strictly controlled environment? How would you account for variations in different sites /locations/ and plants when you speak of "ideal"?

You also mentioned the very high concentrations of metals in the flowers of one plant totally above the water level. Please clarify.

Why did you mention that Utricularia might be considered to be a good indicator of dissolved heavy metals when Pontederia flowers had high concentrations?

BOSSERMAN, R.W: Pontederia flowers in Okefenokee Swamp had high concentrations of Cu, Zn, Mn and Co. This result was based on one sample although Cowgill found a similar phenomenon. These results indicated that there may be an anomaly with Pontederia flowers, because the flowers of most plants have low concentrations of heavy metals. There are scarce data to make a concrete statement about Pontederia's ability to accumulate metals.

Utricularia could be a good indicator of metals because it is rootless and floats in the water. The plant is completely immersed in the water and obtains all of its elemental content from the water. Elemental concentrations were high in Utricularia, indicating that it has a great ability to accumulate the metal. Therefore Utricularia may be a good bioindicator.

WEIS, P: Your Utricularia uptake of Hg is higher than anything we observed in an area downstream from a mercury dump. This might relate to its carnivorous life-style, which places it higher in the food web.

BOSSERMAN, R.W: The Utricularia samples were taken over a period of a year, and the highest concentrations were obtained during a drought which had reduced the water content. Utricularia seems to have several mechanisms for uptaking elements which are not well understood.

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HEAVY METAL CONTENT IN MACROPHYTES FROM PONDS SUPPLIED WITH POST-SEWAGE WATER

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INTRODUCTION

Several authors (Seidel 1966, Culley and Epss, 1973, Volverton and McDonald 1976) point to the possibility of using plants as biological filters for water purification, among others, for removing the heavy metals.

The present paper is a part of broader one on the possibility of using the system of troughflowing ponds with a specific biocenosis in treatment of post-sewage water (sewage after mechanical and biological treatment).

The aim of the paper has been to determine the role of various ecological groups of plants - helophytes (Schoenoplectus lacustris (L.) Palla, Typha latifolia L., Glyceria maxima (Hartm.) Holmb.), pleustonic plants (Lemma minor L. and L. gibba L.) and elodeids (Potamogeton lucens L. and Ceratophyllum demersum L.) in cycling of heavy metals in ponds supplied with post-sewage water.

AREA AND METHODS

The studies were conducted between June and September 1978 in a sewage-treatment plant at Pruszków, near Warsaw, where a model group of troughflowing ponds had been built on an area of about 1 ha. The group consisted of four ponds differing in size and shape but joined together in a row (Fig. 1). The ponds were mostly shallow, with a depth of 0.6 - 0.7 m and about 1.2 m from the banks. Ponds A and B were divided by a barrier, each with a passage, the others being linked by outlet boxes.

The chemical composition of the inflowing post-sewage water as well as the water flowing out of ponds A, B, C and D was investigated monthly. According to Hermanowicz et al. (1976) determined were pH, iron, manganese, zinc, copper, lead, chromium and nickel.

Samples of Glyceria maxima, Lemna minor and L. gibba were taken in June, July, August and September, samples of other species were taken once in August. The concentrations of iron, manganese, zinc, copper, lead, chromium and nickel were determined by atomic absorption spectrophotometry.

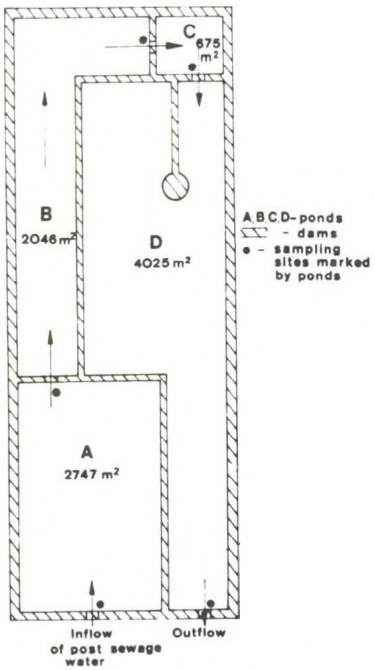

Fig.1. Diagram of the pond complex at the sewage-treatment plant in Pruszków, near Warsaw.

RESULTS

Chemical composition of water

The water in ponds had a slightly alkaline pH (7.2 - 7.6). The content of heavy metals in post-sewage water differed considerably in successive months of investigations as illustrated by 95% confidence limits. The mean content of the majority of metals in water (analysed between June and September) decreased in successive ponds (Fig. 2). Concentrations of Cr and Pb decreased considerably already in pond A, Cr concentration in water running out of pond A was reduced by 90% as compared with the concentration in the inflowing post-sewage water. Reduction of Zn, Cu and Ni took place gradually in successive ponds, whereas the concentration of Fe almost did not change at all, and Mn concentration even increased (Fig.2).

Similar changes in concentrations of heavy metals were observed in the water of successive ponds all the year round (Klekot and Misztak 1981).

The contents of heavy metals in sediments, similarly as in water, decreased in successive ponds. And so, when comparing the metal content in ponds A and B, it was found that Pb decreased from 900 to 130 ppm, Zn - from 3607 to 373 ppm, Cr - from 737 to 81 ppm, Cu - from 134 to 31 ppm, Ni - from 245 to 94 ppm, whereas Mn and Fe had an approximate level in all ponds (Bojakowska and Klekot 1980).

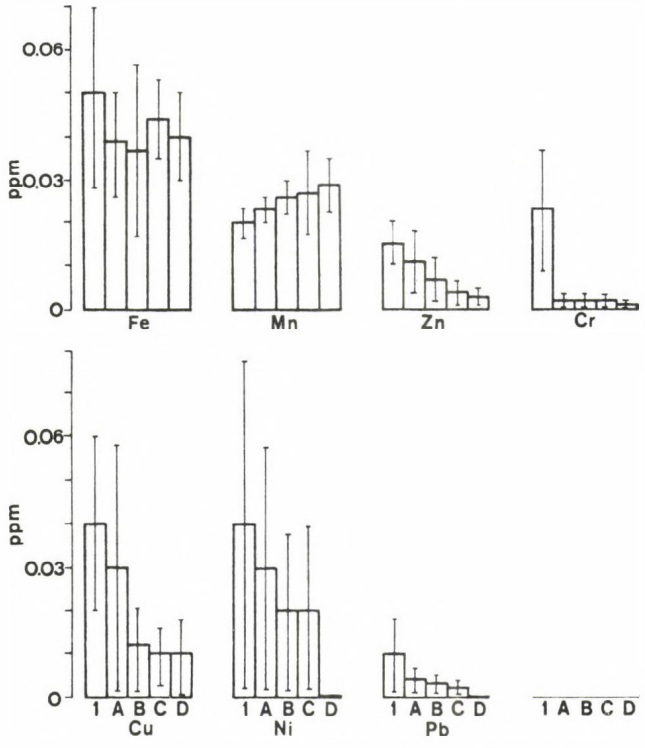

Fig.2. Mean content (±95% confidence limits) of heavy metals (June-September) in inflowing post-sewate water (1) and in water running out from successive ponds (A, B, C, D).

Characteristics of vegetation

Lemna minor and L. gibba occurred in all ponds. They were found on water surface between April and November, with maximal values in August (90% of pond A, 100% of ponds B and C, and only 20% of pond D).

The duckweeds attained the highest dry weight · 1 m^{-2} also in August: 50 g in pond A, 60 g in pond B, 100 g in pond C and 30 g in pond D. Lemna gibba was about 15% of total biomass.

The highest contribution of young fronds was observed between May and July, since August the percentage of older fronds increased considerably.

Glyceria maxima grew mainly in pond A forming a compact belt around its banks and covering 30% of water surface in the close vicinity of the outlet of post-sewage water. G. maxima developed ind pond A abundantly attaining the mean height of 1.6 m. In July about 70% of all G. maxima shoots were in full bloom and fructified in July and August.

The biomass of aboveground parts of G. maxima was in August 1.5 kg dry weight · 1 m^{-2} of overgrown surface area.

Submerged plants did not occur in the three first ponds because of very bad light conditions.

A detailed description of vegetation in ponds analysed is given by Ozimek and Klekot (1979).

In order to compare the absorption and cumulation of heavy metals in different ecological groups of plants in pond A planted were: Potamogeton lucens, Ceratophyllum demersum, Typha latifolia and Schoenoplectus lacustris.

Contents of heavy metals in plants

Concentration of heavy metals were analysed in plants from ponds varying as to metal contents in water (Fig.3). Such a comparison was only possible for Lemna minor and L. gibba as these two species occurred only in all ponds.

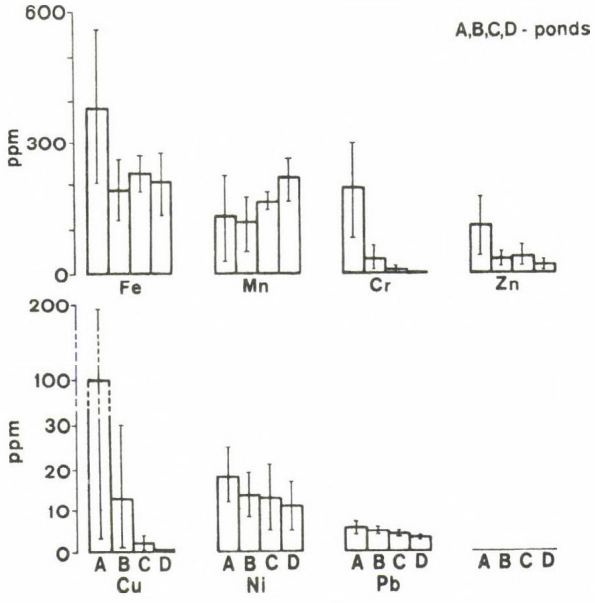

Fig.3. Mean content (±95% confidence limits) of heavy metals (June-September) in duckweeds from successive ponds (A, B, C, D).

The highest concentration of heavy metals, with the exception of manganese, were recorded in duckweeds from pond A. Changes in metal contents in plants from successive ponds varied. Concentrations of Cu and Cr decreased almost ten-fold when comparing plants from ponds A and B, and almost hundred-fold when comparing plants from ponds A and D. Contents of Fe and Cu were only twice higher in duckweeds from pond A as compared with other ponds, and concentrations of Ni and Pb did not change significantly in plants from successive ponds (no statistically significant differences were observed at confidence limits 0.05). The Mn content in duckweeds increased similarly as in the water of successive ponds (Fig.3).

Changes in the contents of heavy metals in Lemna minor, L. gibba and in aboveground parts of Glyceria maxima over the four months of the vegetation season are shown in Figure 4.

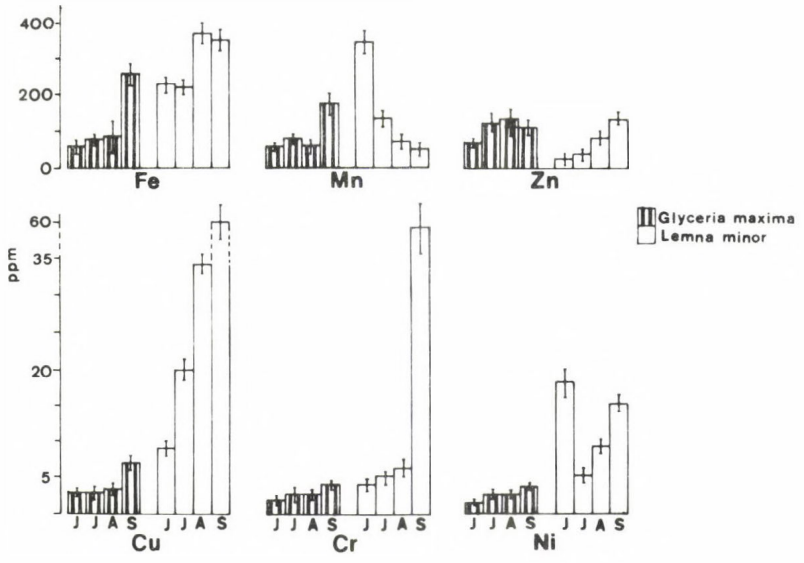

Fig.4. Changes of heavy metals content in Lemna minor, L. gibba and Glyceria maxima from June to September 1978 (pond A).

The concentration of metals in aboveground parts of Glyceria maxima, between June and August, remained more or less on the same level and increased only slightly in September; Fe - three times, Mn and Cu - twice. The concentrations of other metals did not change significantly during the period examined.

A gradual increase in duckweeds was observed between June and September of Zn (30-130 ppm), Cu (8-60 ppm), whereas manganese decreased (340-50 ppm). As regards other metals Ni fluctuated, whereas Cr remained on a similar level during June, July and August to increase rapidly to 38 ppm in September. The data in Figure 5 are for the duckweeds from pond A, but identical changes in concentrations of heavy metals were recorded between June and September in other ponds.

Differences in the contents of metals in plant samples (Glyceria maxima, Lemna minor and L. gibba) taken in the same month were small.

The contents of heavy metals were compared in 7 species belonging to different ecological groups: Schoenoplectus lacustris, Typha latifolia, Glyceria maxima representing the helophytes (submerged macrophytes), Lemna minor, L. gibba - pleustonic plants (floating macrophytes), Potamogeton lucens and Ceratophyllum demersum - elodeids (submerged macrophytes) (Fig.5). All species mentioned were from the same environment (pond A) and were sampled at the same time (August 1978).

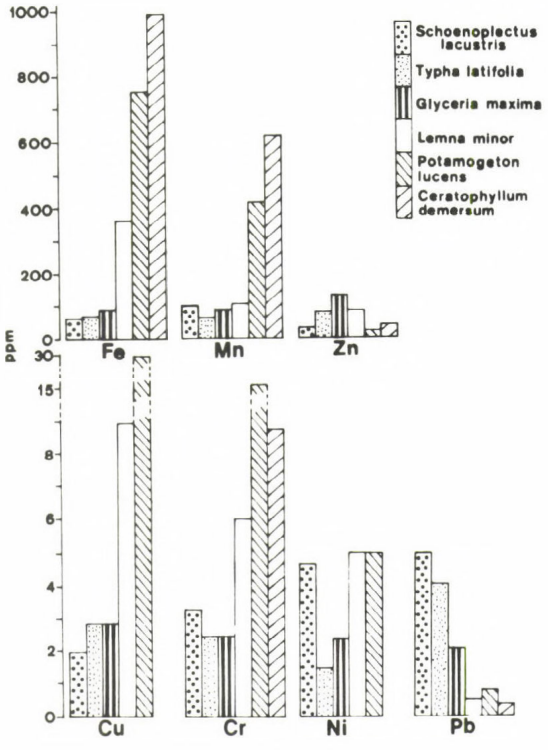

Fig.5. Comparison mean o f heavy metals content in different species (15 August 1978, pond A).

The contents of Fe, Mn, Cu and Cr were the highest in submerged macrophytes and the lowest in aboveground parts of emergent plants. In three species of helophytes these metals occurred at a similar level. No regularity was observed for the relation between Zn and Ni concentrations and the ecological group. Differences within the helophytes were equally big as when comparing the helophytes with elodeids and pleustonic plants. Pb concentration in emergent plants (Schoenoplectus lacustris) was similar to that in floating plants (Lemna minor, L. gibba), but it was much lower in submerged plants (Fig. 5).

DISCUSSION

Plants in ponds supplied with post-sewage water find good living conditions (apart from submerged macrophytes, which suffer from bad light conditions), grow well and attain high biomass. No disturbances in their phenology have been observed (Ozimek and Klekot 1979).

The amount of heavy metals brought to ponds with post-sewage water is small compared with their contents in raw untreated sewage water, where their contents are in thousands of ppm (Kabata-Pendias and Pendias 1979). In the ponds examined heavy metals occur in a gradient, their concentrations decrease (with the exception of manganese) together with the distance from the outlet of post-sewage water. The greatest decrease in their concentration occurs in the first pond, where they are precipitated, adsorbed on seston particles and taken by vascular plants (phytoplankton in these ponds is very poor). With the exception of the first pond, concentrations of metals in other ponds both in water and sediments reach values recorded in natural, not polluted aquatic environments (comparison with data of Kabata-Pendias and Pendias 1979, Bojakowska and Klekot 1980).

Together with the decreasing gradient of metals in water their content in pleustonic plants decreases. But the decrease in metal concentration in water does not always correspond to that in plants, and so despite a slight decrease of copper concentration in water it decreased rapidly in plants, chromium had a rather similar level in the water of successive ponds, whereas its gradient distinctly decreased in plants, nickel decreased rapidly in water between the third and fourth ponds and this decrease was not reflected in plants.

These results show that apart from passive absorption of heavy metals by duckweeds (pointed out also by Hutchinson 1975) they can assimilate some metals selectively. Contents of heavy metals in duckweeds were several hundreds higher than their contents in surrounding water.

Together with the ageing of plant populations analysed the contents of heavy metals increase, and in pleustonic plants this increase is stronger than in aboveground parts of helophytes. Similar changes in time of concentration of zinc, copper and lead in aboveground parts of Phragmites australis (Cav.) Trin. have been observed by Larsen and Schierup (1981).

At the end of the vegetation season only manganese is withdrawn from plants to the environment. Generally manganese is a mobile element in plants and accumulates mainly in young parts of plants. It is transported both as Mn^{+2} cations and in the form of organic complex compounds (Tiffin 1972, Van Goor and Wiersma 1976). Other metals, e.g., chromium, form usually high-molecular compounds of a low mobility and accumulate most frequently in older plant parts (Blincoe 1974), which explains their increase in Lemna at the end of the vegetation season. Pleustonic plants may be of great significance in retaining some metals by means of decaying and falling to the bottom, thus immobilizing them in sediments.

The contents of heavy metals were compared in different ecological groups of macrophytes, which, among other things, differ in vegetative parts absorbing them and in place from where they draw them. Helophytes draw them from the interstitial water of sediments by means of a well developed root system, pleustonic plants take them directly from water and submerged macrophytes from water

either through leaves, e.g., Ceratophyllum demersum which is usually unrooted, or through leaves directly from water and through the root system from sediments, e.g., Potamogeton lucens.

In sediments of ponds the contents of heavy metals exceeded thousand times their contents in water, and thus it should be expected that their contents would be the highest in emergent plants which draw the metals from the substrate. But the contents of the majority of metals (Fe, Mn, Cu, Cr) were the lowest as compared to pleustonic and submerged macrophytes. Does this prove their smaller ability to take and accumulate heavy metals? First the whole pool of heavy metals in sediments does not occur in compounds accessible to plants. Secondly, the present analysis concerns only aboveground parts of helophytes and as it has been mentioned by Schierup and Larsen (1981) Brix and Lyngby (1982) the majority of heavy metals are accumulated mainly in roots.

The considerably high Pb content in emergent and floating macrophytes as compared to the submerged ones can be explained by the fact that both helophytes and pleustonic plants are in danger of lead fall from the atmosphere, which especially in extremely industrialized areas may be its significant source. Such a direct fall is not dangerous for plants constantly submerged in water.

Summing up, it can be said that pleustonic plants should be first of all reccommended for removing heavy metals from water. They are almost equally effecient as submerged macrophytes. They grow well in polluted environments and are not limited by light conditions, which is frequently an obstacle in polluted environments. They draw heavy metals directly from water, they do not set in motion the metal deposit in sediments (as, e.g., emergent macrophytes and some submerged ones), they can be easily removed from the environment.

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DISCUSSION

LORCH, D: You found high concentrations of lead in the emergent parts of Schoenoplectus. Did you wash the plant material prior to analysis to remove atmospheric input /particles/?

OZIMEK, T: Yes, I wash the plants, but it may be that some amount of lead from atmospheric input remained on the leaves of investigated plants.

SALÁNKI, J: If one wishes to use macrophytes to eliminate heavy metals from the water, one should remove these plants when they accumulate the highest amount of metals. Otherwise they will release the metals after the vegetation period again into the water /or bottom/ and no purification takes place . Do you have any plan to do this in practice?

OZIMEK, T: The plants used for water purification must be removed from the water when the concentration of heavy metals is low and replaced by new plants. It is possible by using duckweeds which have a sort phenological cycle and obtain a high biomass in a short time / about 2-3 weeks /.

These plants may be a valuable animal fodder /see Culley and Epss 1973/. The plants with high concentration of heavy metals may be used to produce for example bio-gas. This problem has been investigated by Volwerton /1976/.

WACHS, B: If we will gather the contaminated macrophytes and take them out of the ecosystem of ponds, I believe it will play no role for the metal concentrations in the whole system. Our investigations on the biogenic retention of heavy metals in running waters have shown that the whole biomass is only able to retain maximally 0.02-0.3 % of the metal concentration of the water flow.

JANAUER, G: Are you planning or have you succeeded in putting up a kind of greenhouse for covering the pleustophytes during winter?

OZIMEK, T: I think that duckweeds are very useful in water purification and they can be cultivated throughout the year in greenhouse. May be in the future I will try to use such a system.

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ACCUMULATION, TOXICITY AND LOCALIZATION OF LEAD IN CRYPTOGAMS: EXPERIMENTAL RESULTS

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INTRODUCTION:

The pollution of our environment with an ever larger number of toxicants has during the past decades led to increased efforts to investigate their action on living organisms.
Heavy metals have been the object of many studies since they are persistent and belong to the most widely dispersed industrial pollutants. Several aims have been pursued such as the impact of heavy metal pollution on natural ecosystems and the search for reliable bioindicators. In laboratory experiments at first questions of the metal accumulating capacities of diverse species were of primary importance. At present however investigation of the factors affecting metal accumulation, accumulation mechanisms as well as localization and effect of heavy metals on metabolism and subcellular architecture of the cells have come to the fore.
The paper presented here is an effort to give an abreviated overview of the results of studies undertaken at the Institut für Allgemeine Botanik of the University of Hamburg pertaining to metal accumulation, toxicity as well as localization and ultrastructural changes observed. It is intended to make available data widely scattered in diverse publications as well as data not yet published. It is certainly not intended to give a comprehensive overview over the immense field of literature on heavy metals.

MATERIALS AND METHODS:

Materials used and methods employed have been described in the publications cited. In general the plants used for experiments were cultivated in artificial media either as batch cultures or for specific experiments as homocontinuous or synchronous cultures. Where cultivation was not feasible, material was collected from sites with low background contamination (lichens).
Lead was usually added in the form of aquous stock solutions of the chloride in appropriate amounts. As precipitation occured an ion reduced medium free from sulphate and phosphate was employed. For metal analysis the plant material was washed, lyophilized, wet ashed and the metal content determined using atomic absorption spectroscopy. Preparation for electron microscopy was carried out in the usual way with the samples embedded

in Epon 812 and poststained with uranyl-acetate and lead-citrate.

RESULTS:
Metal accumulation by algae and other aquatic cryptogams is influenced by a number of abiotic and biotic factors which interact with the algae and result in a complex relationship determining metal accumulation and giving rise to a number of consequences concerning metabolism, structure, growth and reproduction of the organisms concerned.

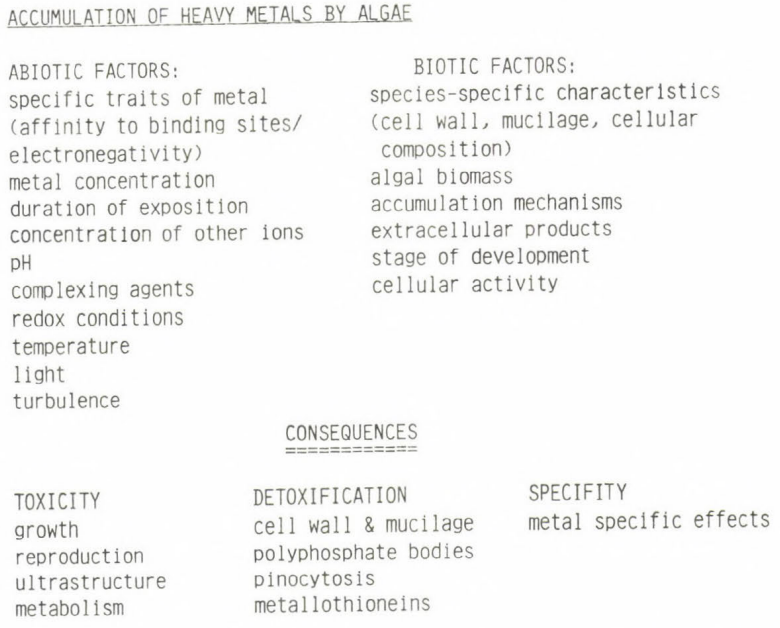

Fig. 1: Some factors affecting metal accumulation by algae and their response to the increased metal burden.

Influence of the external lead concentration:

The plant material was exposed under batch conditions to external lead concentrations ranging from 0 to 5.0 mg Pb/l until equilibrium was reached. In most cases saturation kinetics were observed. The different species varied in saturation level and in the external concentration at which saturation occured. In Fritschiella tuberosa saturation was reached at concentrations above 2.5 mg Pb/l. (Ahlf, 1978 and Ahlf et al. 1980). For Chlorella fusca (Irmer 1982) an external concentration of 4.1 mg Pb/l was necessary. Both, Physcomitrella patens and Cladonia arbuscula reached saturation on application of about 3.0 mg Pb/l. The alga Tribonema aequale did not give distinct saturation kinetics but showed a linear correlation between external lead concentrations up to 2.0 mg Pb/l and the amount of metal accumulated and non linear increase at higher concentrations. With the moss Sphagnum squarrosum and the lichens Evernia

prunastri and Usnea spec. a linear correlation was found over the whole range of concentrations (Lorch, 1984). This relationship also holds true for Chlamydomonas reinhardii (Ahlf et al., 1980).

Influence of duration of exposition on lead accumulation:

In most of the organisms examined time dependent lead accumulation follows saturation kinetics, differences occuring only in the metal content finally found and the time elapsed till equilibrium is reached. These experiments have been described in detail by Ahlf (1978), Ahlf et al. (1980), Christlieb and Weber (1980), Irmer (1978) and Lorch (1977a, 1984). In the case of the lichens Cladonia arbuscula and Usnea spec. however metal accumulation continued during the whole experiment. This was most noticable in Evernia prunastri where a linear dependence of the amount of lead accumulated and the time elapsed was found (Lorch, 1984).
Short time experiments (Irmer, 1982; Ahlf et al., 1980) have shown that in Chlorella fusca approximately 80% of the final lead content are reached within 15 minutes, in Chlamydomonas reinhardii and Fritschiella tuberosa 30 minutes are needed. Extremely fast accumulation of lead can be observed in Tribonema aequale (Lorch, 1984). Here accumulation is almost instantaneous after addition of lead to the algal suspension. This result seems to indicate that the primary step in lead accumulation is of physical nature and probably consists in the adsorptive binding of the metal to components of the cell wall. In some instances, as observed in Microthamnion kützingianum some hours after addition of lead a decrease in the initially accumulated amount of metal can be observed (Lorch, 1977). This is believed to be due to the release of extracellular products by the algae and subsequent complexation of the metal. An unusual time course of lead accumulation is observed in Sphagnum squarrosum (Lorch, 1984). A fast primary accumulation step leads to concentrations of about 800 µg Pb/g biomass within 20 minutes of exposure under standard experimental conditions. This is followed by a release of the accumulated metal in the course of the next 2 to 3 hours with a subsequent reduction of lead content to about 460 µg Pb/g biomass. Further exposition again leads to increasing lead concentrations within Sphagnum squarrosum until equilibrium is reached at an intermediate level. This time course of lead accumulation may be explained by the high ion-exchange capability of Sphagnum and the marked proton secretion of this organism during nutrient accumulation.

Influence of complexing agents on lead accumulation:

In the usual nutrient media, addition of lead leads to the formation of precipitates and thus lead is no longer available for accumulation by the experimental organism. However on separating the plant material from the experimental medium either by zentrifugation or filtration these precipitates are also collected and on analysis accumulation values are obtained which are too high. Precipitation can be prevented by omitting phosphate and sulfate ions from the experimental medium. When no complexing

agent is added lead is predominantly available in the ionic form. When Fritschiella tuberosa is exposed to 500 µg Pb/l after 1 h 1250 µg Pb/g algal dry weight is found. On the other hand precipitation of lead can be prevented by adding the metal as an EDTA complex. If the ratio of EDTA to lead is 1:1 under similar experimental conditions only 490 µg Pb/g algal dry weight are found in Fritschiella tuberosa. Doubling the amount of EDTA further reduces lead accumulation to 66 µg Pb/g algal dry weight. It is presumed that lead accumulation follows the partial decomposition of the complexing agent in the light. In experiments with lead offered in the ionic form no influence of illumination on lead accumulation was observed. Similar results were obtained by Ahlf et al. (1980) using Chlamydomonas reinhardii.

Influence of the pH-value and temperature on lead accumulation:

The primary step in lead accumulation is generally held to consist in the adsorptive binding of the metal ion to negatively charged groups of cell wall and mucilage. It is therefor to be expected that the pH influences metal accumulation. This was shown in experiments with Chlamydomons reinhardii: At pH 5.0 lead accumulation was by higher than at pH 6.6 when lead was applied in the ionic form. In a similar experiment but using lead complexed by EDTA drastic differences in accumulation were observed. At pH 5.0 after only 30 minutes of exposure 50 % of the lead accumulation found after 48 hours had been effected, at pH 6.6 however less than 1 %. An explanation for these results given by Ahlf et al. (1980) might lie in the influence of the pH on the stability constants of EDTA-metal complexes. In these experiments also a distinct influence of the temperature on lead accumulation was observed. At 30°C lead accumulation is approximately double of that at 5°C when the metal is added as EDTA-complex. Addition of lead in the ionic form only shows a slight enhancement (appr. 11 %) of accumulation at 30°C. Similar results were obtained by Ahlf et al. (1980) using Fritschiella tuberosa. Generally lead accumulation increased with increasing acidity of the experimental medium. This is in opposition to the results obtained using Chlorella pyrenoidosa and Pediastrum tetras, as shown in Fig. 2.

In these experiments a pH controlling device was used to establish a pH gradient. Analysis of samples taken at the intervals indicated gave the accumulation curves represented in Fig. 2. Whether the deviating results depend on the special cell wall composition encountered in Chlorella (sporopollenins) and Pediastrum (silica) or on experimental conditions has not yet been fully cleared. It is however interesting to observe that lead binding is at least partially reversable.

In addition to the abiotic factors concerning the environment of the algae, the algae themself influence metal accumulation in a number of ways either throug their specific characteristics, by the number in which they are present (biomass) or by the products they excrete.

Of the factors given in Fig. 1 only the influence of biomass on lead accumulation shall here be more closely examined.

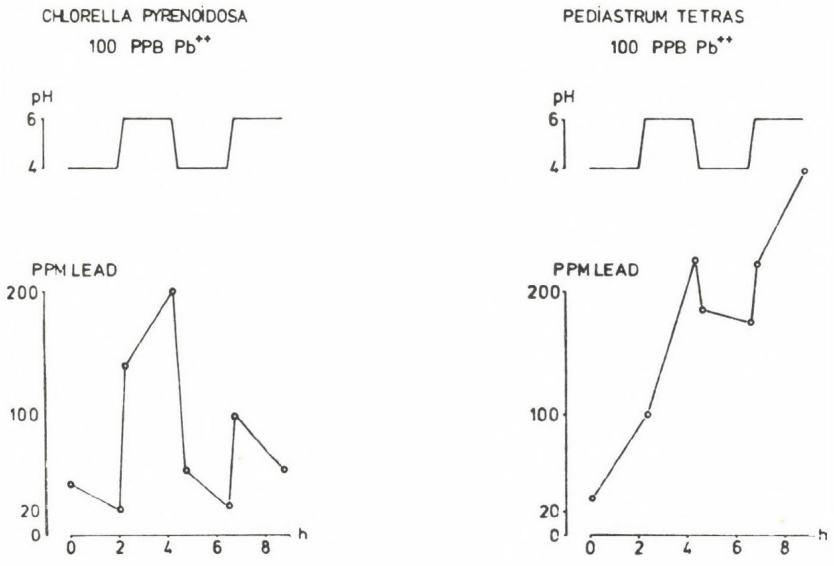

Fig. 2: Influence of pH on lead accumulation by Chlorella pyrenoidosa and Pediastrum tetras under pH controlled conditions.

Influence of algal biomass on lead accumulation:

When algae mosses or lichens are exposed to lead in the ionic form under batch conditions a certain percentage of the metal is removed from the experimental medium and bound to the biomass. The percentage of lead removed and the concentration of the metal found in the organisms depends on the species employed and the biomass utilized. In experiments using 100 µg Pb/l and adding different biomasses (based on dry weight) of Fritschiella tuberosa, Ahlf et al. (1980) were able to show that increasing the biomass resulted in decreasing metal concentrations in the algae. Decreasing the biomass naturally had the inverse effect. However at very low biomasses, with Fritschiella tuberosa at less than 1.6 mg of algal dry weight/ 100 ml, a disproportionate increase is observed due to toxic effects causing death of the algae and probably making more metal binding sites accessable. Depending on the biomass employed, variations in accumulation factors of up to 25 times could be observed. Similar results were also found for Chlamydomonas reinhardii. In short time experiments with Tribonema aequale a

corresponding relationship was found: 4.7 mg algal dry weight/ 100 ml resulted in lead concentrations of 890 µg Pb/g, using 8.1 mg the concentration was lowered to 563 µg Pb/g (Lorch 1984). The decrease in lead concentration with increasing biomass was not only observed in all algae examined but also in mosses and lichens. A representative graph is shown in Fig. 3.

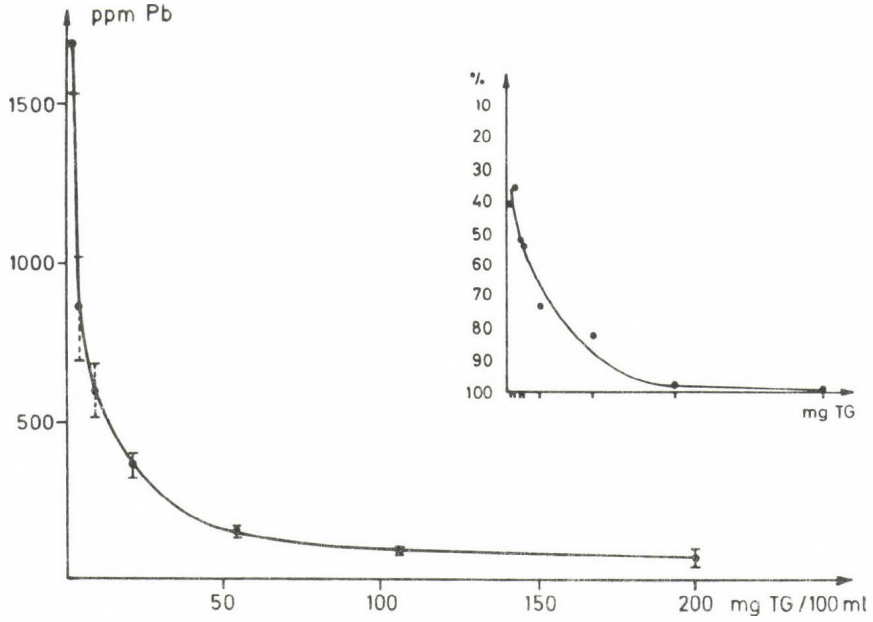

Fig. 3: Lead accumulation by Usnea sp.: Influence of the biomass on the lead concentration found in the lichen. Inset shows the percentage of available lead accumulated by Usnea. (Lorch 1984)

It has been found that different species however differ in the amount of lead they can accumulate under the experimental conditions employed. For this reason. Lorch (1984) suggested that the biomass necessary to bind 50% of the lead under standard conditions in laboratory experiments might be used to characterize the accumulation efficiency of the organism. In this way two distinct groups were found. Sphagnum squarrosum, Physcomitrella patens and Tribonema aequale are efficient lead accumulators with biomasses ranging from 2.0 to 3.2 whereas Usnea sp., Evernia prunastri and Cladonia arbuscula are less efficient needing biomasses of 7.4 to 14.1 mg. It should be noted, that in biomonitoring usually concentration factors are given to describe metal accumulation. In the case of lead, the influence of the biomass employed, can at least under laboratory conditions, not be neglected. Factors reported in which the biomass employed is not given should be used critically.

Consequences of lead accumulation:

Influence of lead on growth, yield, metabolism and ultrastructure:

In long term experiments, using lead complexed by EDTA, Wettern et al. (1976) detected no significant reduction in yield on addition of up to 1.5 mg Pb/l to axenic cultures of Pediastrum tetras. Similarly Netrium digitus was not affected by lead-EDTA concentrations of up to 3 mg Pb/l. In other desmids however significant reduction in yield was observed. Yield was decreased by 50% in Gonatozygon aculeatum and Penium spirostriolatum, by 70% in Closterium ehrenbergii and by as much as 86% in Micrasterias rotata (Lorch 1978a). With Microthamnion kuetzingianum extremely high lead concentrations (80 mg Pb/l as EDTA complex) had to be employed to get as significant reduction in yield after 45 days of culture (Lorch 1974). Due to precipitation long term experiments with ionic lead were not possible.

In short time experiments (up to 48 hours) the effect of lead offered in the ionic form was drastic. Irmer (1982) showed that even 1 µM of lead reduced growth of Chlamydomonas reinhardii by 47% within 48 hours, chlorophyll biosynthesis by 26% and photosynthetic oxygen evolution by 39%. Similar toxic effects were observed in Chlorella fusca. Here however reduction in yield by 37% occured at an external lead concentration of 20 µM. Chlorophyll biosynthesis was reduced by 28% at 5 µM and photosynthetic oxygen production by 49% at 20 µM of lead.

Concurrent with these metabolic changes, exposition to 5 µM of lead caused drastic ultrastructural changes in Chlamydomonas reinhardii. The chloroplast membranes were damaged giving rise to a fingerprint like arrangement. Pyrenoids showed signs of dissolution as did the mitochondria. In the nucleus the nucleolus seemed to dissolve, the nuclear envelope was swollen resulting in a large perinuclear space.

Localization of lead:

Preliminary experiments with strains of Eremosphaera viridis which differ in diameter (Christlieb und Weber 1980) have shown that the cell surface area correlates well with the amount of metal accumulated. The metal load was calculated to be 1.27×10^{-5} ng/µm² for large and 1.32×10^{-5} ng/µm² for small cells. A similar relationship was found by Irmer (1982) using cells of Chlorella fusca obtained from synchronous cultures differing in size. Both, small and large cells had the same lead load when calculated on the basis of the cell surface area.

Proof that the cell wall plays an eminent role in metal accumulation was obtained using isolated cell walls of the desmid Gonatozygon aculeatum. The walls were isolated and analyzed according to Lorch (1978a, b) and Mix et al. (1982). These cell walls can accumulate up to 6% of their dry weight of lead from an experimental medium. Treatment of the cell walls with EDTA solution prior to incubation removes previously adsorbed ions

and increased the lead binding capacity by more than 25%. If however pectins of the cell wall were extracted prior to incubation, metal binding was reduced by more than 30%.

Direct localization of lead accumulated by algae was undertaken using a Laser Microprobe Analyser (LAMMA 500). In artificially contaminated cells of the desmid Phymatodocis nordstedtiana lead deposits were identified in the cell wall, the chloroplast as well as the nucleus (Lorch and Schäfer 1981a, b). Using Energy Dispersive X-ray Microanalysis Irmer (1982) was able to show that in Chlamydomonas reinhardii lead could be localized in the cell wall, the chloroplast and the nucleus and also in vacuoles, where electron dense deposits were evident. Irmer (1982) also found noticable lead deposits in the eye- spot of the alga.

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DISCUSSION

RIHA, V: 1/ Did you test the influence of adaptation on the uptake of heavy metals by algae?

2/ Did you analyse the amount of metalloprotein /metallothionein/?

LORCH, D: 1/ No, such study was not undertaken.

2/ No analytical results are yet available for our algae.

SCHIBER, J.G: It was pointed out on a graph that chlorophyll biosynthesis was greatly reduced and simultaneously photosynthetic oxygen evolution decreased as a result of the toxic effects of lead /and cadmium/. Is that a direct correlation? Or, could it be a consequence of disruption of some other unaccounted for metabolic process or pathway?

Wouldn't the addition of any other toxic element induce the same results?

LORCH, D: Chlorophyll biosynthesis and photosynthetic oxygen production are decreased on addition of other toxic metals.This is also observed when different herbicides are applied. The underlying mechanisms are certainly different. Possible modes of action of the heavy metals have been given by Irmer /1982/.

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HEAVY METALS AND MINERAL NUTRIENT BUDGET IN *PHRAGMITES AUSTRALIS* AND *TYPHA ANGUSTIFOLIA*

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Common reed Phragmites australis Cav. /Trin./ ex Steudel and cattail Typha angustifolia L. are the most popular representatives of emergent macrophytes in the littoral zone of Polish lakes /Szczepańska 1973/. Both plants form large stands that may strongly influence the turnover of matter in the whole water body /Banoub 1975/. This is particularly true for shallow lakes with a high percentage of shoreline overgrown by emergent aquatic vegetation. Lake Gardyńskie, where the present study was performed, is such a pond-like water body with an area of 82.6 ha and a maximum depth of 11.5 m. Nearly 15% of the lake area is covered by emergent macrophytes. The lake is relatively unpolluted, the drainage basin is mostly afforested. The aim of the present study was to trace heavy metals /namely Cu, Cd, Pb, Co, Mo and Mn/ flow through reed and cattail under the "natural background" conditions. For comparative purposes phosphorus changes in reed were also included in this study.

The analysis of the seasonal changes in concentration of investigated elements in shoots revealed three main types of accumulation dynamics /Fig. 1/. The first type of the accumulation curve is characterized by a sharp maximum in early summer followed by a slow decrease later on during the rest of the season. This type is represented by the accumulation of molybdenum in reed and cattail. In the second type the amount of elements in plant shoots is nearly constant during the whole season. This type describes accumulation of lead, cadmium and cobalt in both analysed plants. The changes of

elements in the third type strictly followed the seasonal changes of plant biomass with a maximum in September. Phosphorus, manganese and copper concentration changes were of that type. Seasonal dynamics of metals is probably connected with the role these elements play in plant physiology. For example a molybdenum known for its participation in reactions of nitrate reduction and aminoacid formation, changed similarly to nitrogen in reed /values of nitrogen dynamics taken from Dykyjova 1979/.

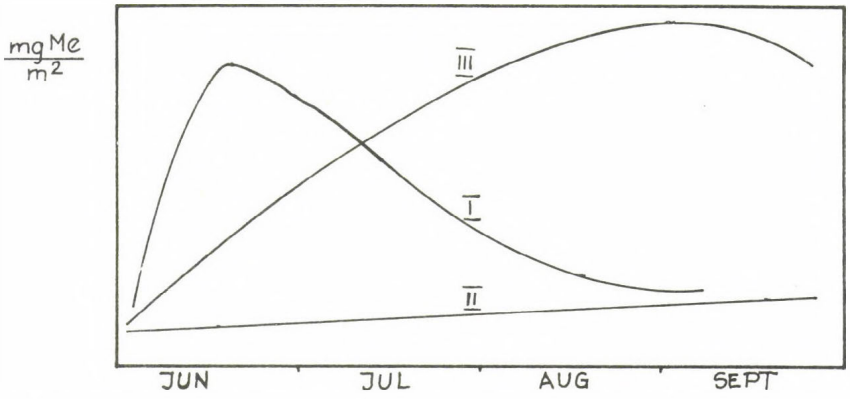

Fig. 1. Three types of heavy metals and phosphorus accumulation in reed and cattail.

Data presented in Table 1 indicate that, besides different seasonal dinamics, heavy metal uptake is, to a high degree, species specific. In connection with the date from Table 1 it should be pointed out that annual uptake is only a minute portion of metals combined in littoral sediments. The ratios of the amount accumulated in shoots to the pool in sediment of 0.5 m depth are for example: for Cu in reed - 10^{-2}, for Pb in cattail - 10^{-4} and for Co in cattail - 10^{-5}.

Underground organs were not analysed during the whole season. Sampling performed once in September showed /Table 2/ that root-rhizomes contain generally more metals than shoots. However, since the former are perennial, their contribution to total turnover of metals in the littoral may be comparable with that of the shoots.

Table 1. Maximum amounts of metals and phosphorus accumulated in plant shoots from square meter of the stand.

plant	elements mg/m^2						
	Cu	Cd	Pb	Co	Mo	Mn	P
reed	1.06	0.40	0.24	1.06	0.27	196.76	960.0
cattail	0.27	0.16	0.07	0.16	1.17	215.42	-

The loss of elements accumulated in plant shoots occurs mainly in two ways i.e. by leaching by rain from living biomass during the vegetative season and after falling down due to the decomposition of plant detritus /authors have no evidence either from the present study or from the literature for the returning of heavy metals and phosphorus from shoots to roots/.

Table 2. Content of heavy metals and phosphorus in underground organs of plants from square meter of the stand.

plant	elements mg/m^2						
	Cu	Cd	Pb	Co	Mo	Mn	P
reed	7.2	0.1	5.3	9.0	1.8	390.6	6000
cattail	0.2	0.1	0.1	0.3	0.6	47.2	-

The process of leaching from living shoots was significant only for lead. Trace amounts of cadmium and phosphorus were leached out from reed, molybdenum from cattail and manganese from both plants /Table 3/. Copper and cobalt were not leached at all. Therefore it seems that, except for lead,the main way of returning heavy metals to the environment is the autumn decomposition of falling shoots. The process /measured by the nylon mesh bag method in natural plant sites/ followed the typical exponential curve /Fig. 2/. Molybdenum from both plants and cobalts, phosphorus and manganese from reed were released faster in relation to the loss of matter from the decomposing detritus. In case of copper, lead manganese and cobalt after the initial intense release, authors observed the enrichment of detritus in these elements /Broken line in Fig. 2/. This was

Table 3. Elements /amounts in $\mu g/m^2$ of stand and percent of actual content in shoots/ leached out from living shoots during one month of vegetative season.

		reed	cattail
Cd	$\mu g/m^2$	0.9	0.0
	%	0.4	0.0
Pb	$\mu g/m^2$	16.9	47.4
	%	7.2	70.0
Mo	$\mu g/m^2$	0.0	6.5
	%	0.0	2.2
Mn	$\mu g/m^2$	362.3	47.4
	%	0.2	0.03
P	$\mu g/m^2$	2300.0	-
	%	0.24	-

probably caused by reabsorption of metals on decomposed material.

As seen from Table 4 nearly 30% of accumulated metals and 46% of phosphorus were released from decomposing reed to littoral water in the early phase of plant decomposition. The respective values for cattail were 3-22% of metals. The rest of the elements gets to the water with a much lower rate in winter and the next season/s/ or is combined in the standing dead shoots of plants.

The analyses of standing overwintering dead plants showed significant amounts of: copper - nearly 140 µg in reed and in cattail, lead - 280 µg in both plants, Mn - 36.8 mg in reed and 24.2 mg in cattail and P - 150 mg in reed, all values per square meter of the plant stand. This material was practically devoid of Cd, Mo and Co. Decomposition of standing dead plant parts depends highly on weather condition, movements of ice cover and other factors leading partitioning and falling down of that material. Thus it is hard to determine the rate of releasing of heavy metals from standing dead plants.

From the elements analysed , special attention should be paid to lead. High percent of lead leached out from living biomass /up to 70% from cattail and 7% from reed/ with a nearly constant amount of metal in shoots during the season

indicate the high turnover rate of lead in the two macrophytes. Moreover, relatively high and increasing /from autumn to spring/ content of lead in standing dead biomass suggests some atmospheric pollution impact on emergent macrophytes.

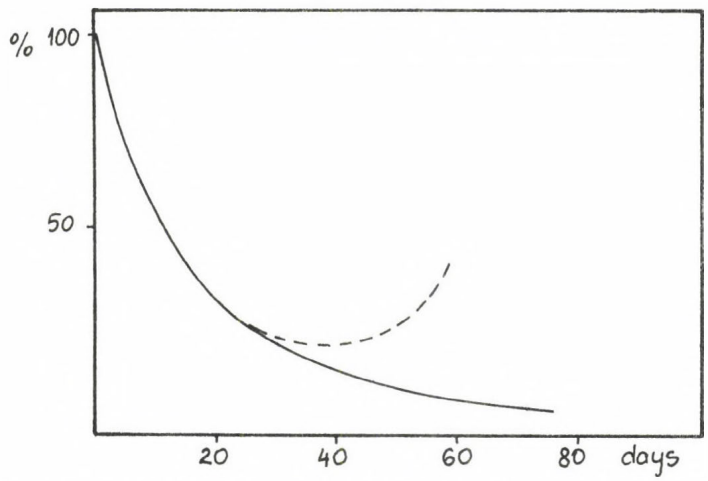

Fig 2. Percent of initial element content in plant detritus during decomposition.

Table 4. The amounts /$\mu g/m^2$ of plant stand/ of elements released to littoral water as a result of autumn plant decomposition / in brackets - percent of maximum accumulation of metal in plant/.

	reed	cattail
Cu	376 /35.5/	60 /22.2/
Cd	86 /21.5/	20 /12.5/
Pb	82 /34.2/	12 /17.1/
Co	313 /29.5/	5 /3.1/
Mo	98 /36.2/	107 /9.1/
Mn	42327 /21.5/	41460 /19.2/
P	443000 /46.1/	-

The significance of emergent macrophytes lies in withdrawing of heavy metals and phosphorus from the substratum and releasing them to littoral water via leaching and decomposition - the process is somethimes called "pumping" /McRoy et al. 1972, Reimold 1972/. Part of the metals released is immediately

combined in situ by sorption on plant detritus and the surface of littoral sediments, this is valid for all analysed elements except the molybdenum and phosphorus. Phosphorus, not sorbed by littoral sediments and easily released by decomposing reed, may thus strongly influence the total nutrient equilibrium in the littoral zone of lakes. Part of the elements may be used up by the littoral primary producers other than helophytes. Due to wave action some of the elements released by macrophytes may reach pelagic waters influencing in such a way the overall turnover of matter in the lake ecosystem.

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THE CONCENTRATION OF MICROELEMENTS IN THE AQUATIC WEEDS OF LAKE BALATON

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The elements present in the water and sediment of Lake Balaton originate either from the geochemical environment or from anthropogenic impact. The elemental content determines water quality and usability and influences the living organisms and their communities. Higher concentrations of heavy metals may prevent self-purification processes. The elements of the lake may be detected in the water, sediment and the plant and animal species.

The advantage of using plants lies in the fact that the macrophytic aquatic vegetation, especially the submerged weeds, are capable of accumulating elements that are found in very low concentrations in the environment.

The water, sediment and plant samples were analysed in the laboratory of the Central Institute of Physics, Hungarian Academy of Sciences.

THE MICROELEMENT CONTENT OF THE WATER OF LAKE BALATON

Based on the analysis of nine water samples the following elements were detected: U, Pb, Ho, Gd, Pr, Ce, La, Ba, Cs, Sb, Sn, Mo, Nb, Zr, Y, Sr, Rb, Br, Se, As, Ga, Zn, Cu, Ni, Co, Fe, Mn, Cr, V, Ti, Al, F, B /cf. Table 1/. Toxic elements /according to Wood 1974 and Wilson 1979 are underlined in this list.

One source of the elements is the diversified geochemical environment in the watershed of the lake and along its shore. In the south the parent material consists of sand, loess and peat, whereas on the northern side limestone, dolomite, basalt

Table 1. The microelement content of the water of Lake Balaton /mg/l/

Elements	1.	2.	3.	4.	5.	6.	7.	8.	9.
U	0.3	-	1.5	0.15	-	0.32	0.68	2.7	1.5
Pb	-	2.1	1.6	-	-	-	-	3.2	-
Ho	-	-	-	0.072	-	-	0.23	0.16	-
Gd	10.00	0.76	6.7	0.33	2.7	-	2.1	20.00	4.8
Pr	-	-	0.11	0.41	-	0.14	-	0.38	-
Ce	-	-	0.44	-	-	-	-	0.16	-
La	0.091	0.56	0.43	1.5	-	0.50	0.098	0.56	0.16
Ba	10.0	15.0	18.0	1.7	4.2	6.2	8.2	6.6	7.8
Cs	-	-	0.021	-	-	0.018	0.075	0.027	-
Sb	0.37	0.42	0.88	0.46	0.076	-	0.60	1.1	-
Sn	0.64	-	-	-	-	-	-	-	-
Mo	2.1	0.60	3.7	0.65	3.2	2.1	3.4	6.8	2.8
Nb	-	-	-	-	-	0.25	-	-	-
Zr	0.92	-	1.1	-	0.47	-	-	2.0	0.83
Y	-	0.089	0.18	0.039	0.12	0.32	0.25	0.24	0.21
Sr	190.0	64.0	220.0	12.0	86.0	49.0	45.0	64.0	150.0
Rb	0.41	0.094	0.49	0.13	0.42	0.31	0.44	0.31	1.1
Br	8.6	6.6	25	18	8.4	8.8	9.3	19	15
Se	0.21	0.43	0.25	0.070	0.58	0.86	0.61	0.86	0.38
As	0.98	2	4.7	0.82	0.81	2	6.1	6	0.18
Ca	20	4.6	7.1	2.5	4.1	3.1	43	92	11
Zn	0.29	0.21	0.24	0.43	0.28	0.42	0.74	0.45	1.9

Elements	1.	2.	3.	4.	5.	6.	7.	8.	9.
Cu	0.68	0.36	0.38	1.0	0.49	0.44	0.49	0.74	0.49
Ni	0.58	0.90	0.93	0.33	0.80	1.2	0.85	1.8	1.1
Co	0.077	0.16	0.092	0.052	0.32	0.21	1.7	1.2	0.28
Fe	160	120	1900	2.7	23000	9.3	23	70	58
Mn	5.8	4.4	10	0.36	59	0.88	0.93	8.8	1.0
Cr	2.2	1.2	3.9	0.27	9.6	0.44	1.2	2.5	1.5
V	1.3	1.4	3.2	0.11	3.7	0.55	1.4	1.4	3.2
Ti	3	2.3	5.1	0.14	6.2	1.5	1.8	6.6	2.1
Al	71	32	250	0.089	44	22	4.6	9.3	38
P	50	15	59	42	170	10	540	3800	900
B	18	14	14	50	18	37	9.7	91	54

1. Balatongyörök-Szépkilátó
2. Szigliget
3. Badacsony-Badacsonytomaj
4 Balatonudvari
5 Tihany: Bozsai-Bay
6. Csopak: Kerekedi-Bay
7. Balatonalmádi-Balatonkenese
8. Keszthely the middle of Lake Balaton
9. Siófok the middle of the Balaton

and sandstone form the base rocks. Some elements get into the lake through precipitation and various water courses.

THE ELEMENTAL CONTENT IN WEEDS, THE CONCENTRATION FACTOR OF ELEMENTS

The elemental content of submerged weeds in Lake Balaton has been analysed /Kovács 1978, Kovács and Tóth 1979, Teherani et al. 1981/. Heavy metal concentrations in animals are reported by Salánki et al. /1981, 1982/.

The concentrations of rare elements /i.e., microelements and ultramicroelements/ in the more abundant weeds are discussed in the sequel.

The submerged or floating weeds /Ceratophyllum submersum, Hydrocharis morsus ranae, Lemna trisulca, Myriophyllum spicatum, Potamogeton pectinatus, Potamogeton perfoliatus, Stratiotes aloides/ accumulate rare elements in extremely different quantities. The macroelement content of plants is usually characteristic of the species, whereas the quantities of microelements and ultramicroelements /incl. heavy metals/ depend on the environment from wich they are taken up. Therefore, weed species as accumulative indicators may be used in detecting sewage water pollution and characterizing the geochemical environment.

Uranium, which is present in the water, was not detected in the plants. On the contrary, several elements, such as Bi, Dy, Eu, Sm, Nd, I, Ag and Sc were found only in the weeds. Ceratophyllum submersum contains Pb, Ni, Cr, V, Br, As and Ti in large amounts. The presence of Pr, Ce, La, Cs, I, Zr, Y, Ga and Co in the samples collected at Balatonudvari is indicative of the special geochemical environment. The high concentrations of Sb and Cu in the vegetation at Balatonalmádi seem to indicate a pollution of industrial origin. The concentration factor of elements,

$$\frac{\text{concentration in the plant /ppm/dry matter/}}{\text{concentration in the water /}\mu\text{g/ml/}}$$

/cf. Bowen 1956, Morris and Bale 1975, Foster 1976/, is between 10 and 10^5. Cs, Sb, Y, Ni, Co, Cr, V and Ti are accumulated in the largest amounts.

Hydrocharis morsus ranae contains Zn, Ni, Sc and B in relatively high concentrations. As a consequence of industrial process-water /Balatonkenese/ large amounts of Sb, As, Zn and Co are taken up by this plant. Thi highest concentration factor was found to be that of Zn /Table 2/.

In Lemna trisulca, a floating weed common in reed stands, a total of 26 microelements were detected. In large amounts Sb, Sn, Cu, Ti and B are accumulated. The concentration factor of Zn has a magnitude of 10^5 /Table 2/.

The microelement composition of Myriophyllum spicatum is similar to that of Ceratophyllum submersum, large amounts of microelements are accumulated in this plant. Characteristic are the high concentrations of Pb, Nd, I, Zr, Y and V. The samples collected in the Szigliget area contain much Zn and Cu, porobably because fungicides widely used in vineyards around the lake are washed into the water. Zn is accumulated at a magnitude of 10^6, Cs, Co and Ti at 10^5 /Table 2/.

Potamogeton pectinatus accumulates high concentrations of Pb, Br, Ni, Ti, B, Zn and Cu. Zn is taken up from the geochemical environment at the highest magnitude, 10^5 /Table 2/.

The elemental concentrations in the leaves of Potamogeton perfoliatus are twice or three times higher than those in the stem. High concentrations of Pb, Zn and Ti are detected in the leaves, whereas high boron content characterizes the stem. I, Mo, Zr, Ga, Cu, Ni and Co are also present in large amounts.

In case of Stratiotes aloides much Sb, Y, Zn, Cu, Ni, Co and Ti are characteristic of the submerged individuals. The concentration factor of Y, Zn, Co and Ti is 10^5 /cf. Table 2/.

THE MICROELEMENT CONTENT OF PHRAGMITES COMMUNIS, GLYCERIA MAXIMA AND TWO TYPHA SPECIES

In the organs of Phragmites communis, Typha angustifolia, T. latifolia and Glyceria maxima the elements taken up from the sediment and, in case of the adventitious roots of reed, from the water are translocated to different extent.

In Phragmites communis Bi, Ho, Dy, Tb, Gd, Eu, Sm, Nb, Pr are detectable only in the subterranean organs and the

Table 2. The concentration factor of microelements in the submerged weeds

		7	1	2	3	4	5	6
	Bi							
T	Pb	10^3	10^4	10^3-10^4	10^3	10^3-10^4	10^2-10^3	10^2-10^3
	Nd							
	Pr	10^2-10^3	10^2-10^4	10^2	10^3	10^3-10^4	10^2-10^3	10^2-10^3
	Ce	10^2-10^4	10^3-10^4	10^2-10^3	10^3	10^4	10^3	10^3-10^4
T	La	10^2-10^3	10^3-10^4	10^3	10^3	10^3-10^4	10^3	10^2-10^3
T	Ba	10^3		10^4				
	Cs	10^3-10^4	10^3-10^5	10^3		10^4-10^5	10^4	10^4
	I							
T	Sb	10^3	10^3-10^5	10^3-10^4	10^5	10^2	10^3	10^2-10^3
T	Sn	10^2-10^3	10^3	10^3-10^4	10^4	10^3	10^2	10^2-10^3
	Mb	10^2-10^3	10^3	10^3	10^2	10^2-10^4	10^2-10^3	10^2-10^4
T	Nb	10^2-10^3	10^2-10^3	10^3	10^3	10^3-10^4	10^3	10^2-10^3
T	Zr	10	10^3	10^2-10^3	10^2	10^3-10^4	10^2-10^4	10^2-10^3
	Y	10^2-10^5	10^3-10^5	10^2-10^3	10^3	10^4	10^2-10^3	10^3
	Sr	10^2						
	Rb							
	Br	10^3	10^3	10^3		10^3	10^3	10^2-10^3
T	As	10^3	10^4	10^4		10^2-10^3	10^2	10^2
T	Ga	10-10^3	10-10^3	10-10^3	10	10^2-10^3	10^2	10^2
T	Zn	10^4-10^5	10^4	10^5	10^4	10^6	10^4-10^5	10^4-10^5
T	Cu	10^4	10^3-10^4	10^4	10^4	10^5	10^3-10^4	10^4

		7	1	2	3	4	5	6
T	Ni	10^3-10^4	10^4-10^5	10^4	10^3	10^4	10^3-10^4	10^4
T	Co	10^2-10^5	10^3-10^5	10^3-10^4	10^2	10^4-10^5		10^3-10^4
	Fe							
	Mn							
	Cr	10^3	10^4-10^5	10^2-10^3	10^3	10^3-10^4	10^4	10^3-10^4
	V	10^2-10^4	10^3-10^5	10^3	10^3	10^4	10^3	10^3-10^4
	Ti	10^4-10^5	10^4-10^5	10^4	10^4	10^5	10^4	10^4-10^5
	Al	10^2						
	Sc							
	F	10-10^2	10-	10-		10-10^2	10-10^2	
	B	10-10^3	10^2-10^3	10^3	10^4		10^3-10^4	

1. Ceratophyllum submersum
2. Hydrocharis morsus ranae
3. Lemna trisulca
4. Myriophillum spicatum
5. Potamogeton pectinatus
6. Potamogeton perfoliatus
   /L: Leaf, S: Stem/
7. Stratiotes aloides

Table 3. The microelement content of the sediment and the organs of Phragmites communis

Elements	Sediment	Leaf	Stem	Adventitious root	Rhizome	Root	Root lets
Bi	-	-	-	-	-	-	0.1
Pb	6	0.7	0.2	9	0.9	3	5
Ho	0.4	-	-	0.15	0.07	0.08	0.07
Dy	2.5	-	-	0.9	0.7	0.8^{x}	0.4
Tb	0.4	-	-	0.15	0.07	0.1	0.07
Gd	2.5^{x}	-	-	0.9	0.4^{x}	1.5^{x}	0.5
Eu	0.8	-	-	0.3	0.14^{x}	0.4	0.15
Sm	2^{x}	-	-	1.5	0.7^{x}	1.5	0.5
Nd	7^{x}	-	-	3.5	2.5	16^{x}	1.3
Pr	1.3^{x}	-	-	1	0.35	4^{x}	0.2
Ce	4	0.4	0.1	20	2.5	8	2
La	2.5	0.2	0.04	4.5	-	5	0.7
Ba	130	25	3	90	20	50	50
Cs	4	0.2	0.08	3	0.6	0.7	-
J	0.3	0.05	0.15	0.2	3	0.7	0.3
Sb	0.7	0.2	0.05	0.3	0.25	0.3	0.4
Sn	1	0.15	0.1	0.9	0.6	0.5	0.5
Ag	3	0.3	0.1	0.2	0.15	0.4	0.4
Mo	1	0.5	0.15	0.5	0.4	0.3	0.5
Nb	2.5	0.06	0.015	2	0.25	0.5	0.5
Zr	25^{x}	0.5	0.15^{x}	9^{x}	20^{xx}	2.5^{x}	2.5
Y	6	0.2	0.04	4	2	0.5	1.2

Elements	Sediment	Leaf	Stem	Adventitious root	Rhizome	Root	Root lets
Sr	140	70	10	250	50	60	120
Rb	60	20	15	70	70	40	20
Br	0.6	4	2.5	3.5	10	3	2
As	22	10	7	7	35	20	40
Ga	5	0.2	-	4	2	2.5	2.5
Zn	20	18	6	40	6	18	60
Cu	2.5	12	4	20	20	7	10
Ni	15	0.6	0.4	10	1	12	10
Co	2.5	0.08	0.05	3	1.3	8	15
Fe	~7000	~800	~250	+	+	++	++
Mn	700	~400	~100	+	+	+	+
Cr	15	2.5	0.5	30	7	16	10
V	35	1.5	0.2	50	6	18	12
Ti	~3000	60	7	20	~250	~400	~400[x]

~ = informing data; +1000 weight ppm; ++ 10 000 weight ppm; +++ 100 000 weight ppm;

x = the element marked exhibits an inhomogeneous distribution in the sample

xx = significant inhomogeneity observed

Table 4. The microelement content of the organs of Glyceria maxima related to dry matter
Szigliget

Elements	Leaf	Stem	Root
Pb	3.09	7.04	7.05
Nd	0.62	-	0.99
Pr	0.12	-	0.14
Ce	0.25	0.14	0.41
La	0.12	0.07	0.70
Ba	XX	X	XX
Cs	0.37	0.21	1.20
I	0.12	0.07	2.12
Sb	0.37	0.11	0.21
Sn	0.62	0.35	0.70
Mo	1.24	0.35	0.70
Nb	0.15	0.84	0.42
Zr	0.62	0.35	1.76
Y	0.15	0.04	0.42
Sr	XX	X	XX
Rb	X	XX	XX
Br	24.72	7.04	9.17
As	2.47	0.14	0.70
Ga	0.62	0.70	1.06
Zn	98.89	70.42	56.42
Cu	30.90	17.60	17.63
Ni	1.85	0.42	4.23
Co	0.49	0.11	5.64
Fe	XX	XX	XXX
Mn	XX	XX	XX
Cr	1.85	0.56	5.64
V	1.85	0.56	10.58
Ti	4.94	4.22	84.63
Sc	0.07	0.08	0.42
F	0.04	0.08	3.53
B	14.83	4.22	21.16

X = 0.1 0.2 mg %, XX = 0.2 - 1.0 mg %, XXX = 1.0 mg %

Table 5. The microelement content of the organs of Typha angustifolia related to dry matter

Elements	Leaf	Stem	Rhizome	Root
Bi	-	-	-	0.16
Pb	2.91	2.12	-	8.05
Gd	-	-	-	1.29
Sm	-	-	-	3.22
Nd	-	-	-	5.63
Pr	0.06	0.28	0.22	1.61
Ce	0.23	0.28	0.44	6.44
La	0.06	0.14	0.22	2.75
Ba	X	35.38	54.94	XX
Cs	0.11	0.14	0.33	1.29
I	0.23	-	0.88	0.48
Sb	0.35	1.96	0.22	1.61
Sn	0.29	0.71	49.45	7.25
Ag	-	0.42	1.43	-
Mo	0.58	1.77	1.10	4.02
Nb	0.56	0.14	1.10	1.61
Zr	0.29	0.14	1.10	6.44
Y	0.06	0.07	0.22	3.22
Sr	XX	X	XX	XX
Br	X	+	+	-
As	+	+	+	-
Ga	0.29	0.71	1.10	7.24
Zn	20.39	12.03	36.26	128.82
Cu	14.57	8.49	24.17	96.62
Ni	8.74	4.24	1.65	43.48
Co	0.23	0.28	0.44	6.44
Fe	XX	XX	XXX	XXX
Mn	XX	X	XX	XX
Cr	9.90	1.41	7.69	64.41
V	0.87	0.28	1.65	51.53
Ti	34.96	16.28	XX	XXX
F	0.58	0.71	0.55	4.02
B	5.24	12.03	3.84	27.37
Al	XX	24.77	XXX	XXX

+ informing data, X = 0.1 - 0.2%, XX = 0.2 - 1%, XXX = above 1 mg %

Table 6. The microelement content of the organs of Typha latifolia related to dry matter

Elements	Leaf-stem	Rhizome	Root
Pb	2.7	1.21	52.75
Nd	0.43	1.69	14.77
Pr	0.11	0.24	2.11
Ce	0.42	2.42	21.07
La	0.22	1.21	8.44
Ba	X	X	XX
Cs	ny	0.61	6.33
I	1.1	0.121	0.21
Sb	0.22	0.181	0.21
Sn	0.54	0.36	3.16
Mo	1.11	1.21	0.21
Nb	ny	0.14	5.72
Zr	1.29	1.4	25.32
Y	0.23	0.30	12.61
Sr	XX	XX	XX
Rb	X	X	X
Br	21.6	12.1	0.21
As	0.11	0.61	4.22
Ga	0.54	0.30	10.55
Zn	32.4	36.3	316.5
Cu	14.0	72.6	52.75
Ni	1.62	3.6	31.65
Co	0.43	1.8	31.65
Fe	XX	XXX	XXX
Mn	XX	XX	XX
Cr	1.62	0.48	84.4
V	1.62	3.63	73.85
Ti	27.0	72.6	1264.2
Sc	0.12	ny	
F	1.29	ny	0.10
B	12.46	0.18	2.52

adventitious roots developed on underwater noda. The stem and the leaves are free from these elements. Most microelements are charasteristic of the subterranean organs and adventitious roots, the concentrations are also the highest in these organs /Table 3/.

The leaves of Glyceria maxima are particularly rich in Zn, Cu, Br and B, as well as Ba, Sn, Rb, Fe and Mn. Higher concentrations of Pb, Ni, Cr, V and Ti are typical the roots /Table 4/.

In Lake Balaton, similarly to other lakes of Europe, the Typha species increase in abundance as a result of environmental impact.

The subterranean organs /rhizome and root/ of Typha angustifolia and T. latifolia accumulate much more microelements and toxic elements /e.g., Pb, Zr, Zn, Cu, Ni, Co and Ti/ than reed /Tables 5,6/.

SUMMARY

1. The rare elements in the geochemical environment /water, sediment/ can also be detected by analyzing plant samples.

2. In the submerged and floating weeds the concentration factor of some microelements may be as high as 10^5-10^6.

3. In Phragmites communis, Glyceria maxima, Typha latifolia and T. augustifolia most microelements are accumulated in the subterranean organs.

4. As a result of environmental pollution, the two Typha species rapidly spread replacing reed stands. They are capable of accumulating larger amounts of microelements than reed.

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DISCUSSION

LORCH, D: You stressed that Typha angustifolia contains large amounts of lead in the rhizome /root. In our studies we analysed roots of Iris pseudocorus from natural habitats. The roots had been washed free by natural wave action and were free from contaminating sediment particles. In these roots we too observed large amounts of lead.

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LEAD IN A PELAGIC FOOD CHAIN

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The pelagic food chain of the central Pacific exhibits a bioenrichment of calcium with respect to lead in the primary and secondary trophic levels and a biopurification in higher trophic levels. Phytoplankton contains an elevated (800-fold) atomic ratio of lead to calcium ($2x10^{-6}$) relative to that of sea water ($3x10^{-9}$), which contains 6 ng Pb/kg. That ratio is further elevated in zooplankton ($3x10^{-6}$), which have lead concentrations (40 ng Pb/g fw) similar to those of phytoplankton (50 ng Pb/g fw), but lower calcium concentrations. This ratio then declines in intermediate carnivores (anchovies), which contain relatively lower lead concentrations (20 µg Pb/g fw) to $9x10^{-7}$ in top carnivores (tuna), which contain only 0.3 ng Pb/g fw in their muscle tissues. The bioenrichment in phytoplankton is attributed to the preferential sorption of lead relative to calcium by organic chelates on their surfaces, which exceeds the biopurification of their calcareous shells. The bioenrichment in zooplankton is attributed to their digestion of the bioenriched organo-lead complexes of phytoplankton and their elimination of the biopurified calcareous shells of phytoplankton, as well as the preferential complexation of lead relative to calcium on zooplankton surfaces. The biopurification in fish is attributed to their relatively reduced sites for surface complexation and the efficiency of metabolic biopurification processes. While all of the preceding concentrations of lead are lower than commonly reported, they all are at least one order of magnitude above natural concentrations, based on our understanding of the lead cycle in a pelagic food chain and

the contamination of central Pacific sea water by industrial lead aerosols.

Lead fluxes to the marine environment have been perturbed by anthrophogenic emissions of lead (4×10^8 kg/yr), which now exceed natural emissions of lead (2×10^6 kg/yr) by two orders of magnitude (Settle and Patterson, 1980). This contamination, which was initiated over 5000 years ago with the discovery of cupellation (Patterson, 1965), currently reflects the global production of three million tons of lead annually (Nriagu, 1978). It is evidenced by the 300-fold increase of lead concentrations in the Greenland ice cap over the past 3000 years (Murozumi et al., 1969; Ng and Patterson, 1981). It is also indicated by the subsurface maxima in vertical lead concentration ocean profiles which correlate with eolian inputs of industrial lead aerosols (Flegal and Patterson, 1983). The isotopic composition of that lead confirms its anthropogenic origin (Stukas and Wong, 1981; Settle and Patterson, 1982; Flegal et al., 1984).

Lead concentrations in marine organisms also exhibit this perturbation. For example, coastal marine organisms exhibit spatial differences in lead concentrations which correlate with differences in anthropogenic lead inputs (Burnett et al., 1980), and fossil corals exhibit temporal differences which correlate with the exponential increase in industrial lead emissions (Shen and Boyle, personal communication). The data from those marine organisms conform with the data in similar studies of lead contamination in aquatic and terrestrial ecosystems (Elias et al., 1982; Shirahata et al., 1980). They also conform with measurements of lead contamination in man, which indicate that lead concentrations in North Americans are elevated two or three orders of magnitude above natural concentrations to a level that is approaching acute toxicity (Ericson et al., 1979; Settle and Patterson, 1980).

In each of the preceding studies, lead concentrations were normalized to calcium concentrations because of the similarities in their biological uptake, internal distribution and excretion (Patterson, 1965; Hirao and Patterson, 1974; Elias et al., 1976;

Burnett and Patterson, 1979; Schaule and Patterson, 1979). This affinity is due to the chemical similarities of lead and calcium which account for their nearly identical morphological distributions in organisms. Since calcium is a nutrient metal that is transferred along food chains, normalization of lead to calcium provides the most definitive evaluation of lead contamination in different trophic levels.

This normalization was utilized to calculate lead contamination in a pelagic food chain using the lead to calcium atomic ratios of intertidal organisms and pelagic fish, since there were no appropriate data for phytoplankton or zooplankton at that time (Burnett, 1978; Burnett et al., 1980). That model was based on data from an aquatic food chain (Elias et al., 1979), which exhibited bioenrichment of calcium with lead at the primary trophic level and biodepletion at all subsequent levels.

The premise of the model was that the principal reservoirs of both lead and calcium in most organisms are calcareous tissues and that lead is much less efficiently accumulated than calcium in those tissues. Therefore, biodepletion of calcium with respect to lead occurs because the latter is only inadvertently and inefficiently absorbed as a trace constituent of calcium.

However, recent measurements of the lead concentrations of oceanic phytoplankton and zooplankton reveal an additional bioenrichment of calcium with respect to lead at the second trophic level (Flegal and Patterson, 1984), as illustrated in Fig. 1. Phytoplankton contained an elevated ($\sim$ 800-fold) atomic ratio of lead to calcium ($\sim 2 \times 10^{-6}$) relative to that of Central Pacific sea water ($\sim 3 \times 10^{-9}$), which contained 6 ng Pb/kg (Flegal and Patterson, 1983). That ratio in phytoplankton is comparable to that of macroalgae from relatively less polluted coastal waters that were utilized as the primary producer by Burnett and Patterson (1977) in their original food chain model. Conversely, copepod zooplankton contained an even more elevated ratio ($\sim 3 \times 10^{-6}$) which contrasted with the reduced ratio ($\sim 1 \times 10^{-7}$) of the spiny lobster they utilized as the herbivorous zooplankton in that model. This increase in the lead to calcium ratio at the secondary trophic level reflected the lower calcium concentrations of the zooplankton, since their lead concentra-

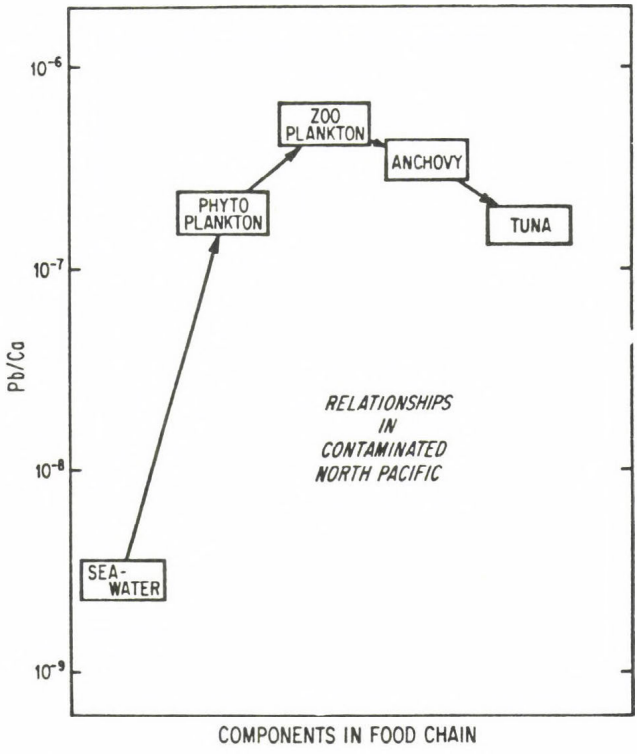

FIGURE 1. Bioenrichment followed by biopurification of calcium with respect to lead in components in marine food chain characteristic of the main biomass in the open ocean.

tions (40 ng Pb/g fw) were similar to that of the phytoplankton (~50 ng Pb/g fw). The ratio sequentially decreased in intermediate carnivores (anchovies), which contained relatively lower lead concentrations (~20 ng Pb/g fw) to ~9 x 10^{-7} in top carnivores (tuna), which contained only 0.3 ng Pb/g fw in their muscle tissues.

The bioenrichment of calcium with lead in phytoplankton is attributed to the factors delineated by Burnett and Patterson (1977). Lead is preferentially sorbed relative to calcium by organic chelates on phytoplankton surfaces. This bioenrichment exceeds the concurrent biopurification that occurs in the formation of their shells of calcerous phytoplankton.

The subsequent bioenrichment in zooplankton is partially attributed to their digestion of the bioenriched organo-lead complexes of phytoplankton and their elimination of biopurified calcareous shells of phytoplankton. Since those shells are not digested, there is a net bioenrichment of zooplankton even though some biodepletion of digested phytoplankton tissues may occur through the metabolic activities of the zooplankton.

The bioenrichment of zooplankton may also be partially due to its preferential sorption relative to calcium by organic chelates on zooplankton surfaces. This was indicated by the elevated lead concentration of gelatinous tunicates (7.4 µg/g dry weight), which was at least twice that of all other zooplankton on a dry weight basis (Flegal and Patterson, 1984). Surface uptake has previously been shown to be the principal source of several radioisotopes.

While the preceding lead concentrations are lower than commonly reported, they are at least one order of magnitude above natural levels. This has been indicated by both ocean lead concentration profiles (Flegal and Patterson, 1983) and fossil coral lead to calcium ratios (Shen and Boyle, personal communication). Since the marine food chain is less efficient in the biopurification of lead than terrestrial systems, marine organisms may now be subjected to comparable or greater levels of sublethal lead toxicity than terrestrial organisms. Therefore, future studies of the lead cycle in the marine biosphere should concentrate on its sublethal effects in the primary and secondary

trophic levels, utilizing the ultra-clean techniques required for accurate lead measurements.

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DISCUSSION

FOWLER, S.V: If Pb contamination is such a problem with environmental samples, is there any validity in making routine Pb measurements for monitoring programmes? In other words, would the signal to noise ratio be too low to detect real changes in environmental levels of lead?

FLEGAL, A.R: The national mussel watch study demonstrated the validity of routine monitoring programs, which employ trace metal clean techniques, to provide a relative measure of lead contamination. Unless such programs utilize ultra-clean techniques they will not provide an accurate measure of lead contamination, and the signal to noise ratio may vary markedly between laboratories and between sampling periods.

SALÁNKI, J: You mentioned the symptoms of lead poisoning in men. Do you have any information on the effect of lead on the irritability, learning ability or behavior on aquatic animals like crustaceans or fishes?

FLEGAL, A.R: This is one of the principal areas of concern. What is needed are carefully controlled biological studies utilizing ultra-clean techniques, so that the responses in organisms may be related to true ambient lead levels.

LORCH, D: What role do fecal pellets play in lead elimination from the oceanic surface layer?

FLEGAL, A.R: The relatively short residence time /1-2 years/ of lead in oceanic surface waters is attributed to its sequestering by particulates, such as fecal pellets. Scott Fowler is specifically investigating this as part of the VERTEX study.

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HEAVY METAL CONTENT OF THE FISHES OF LAKE BALATON, DANUBE AND TISZA DURING THE PERIOD OF 1979-1982

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ABSTRACT

A study was performed in 1979-1982 on the heavy metal content of some characteristic species of Lake Balaton, the Danube and the Tisza river, i.e. barbel /Barbus fluviatilis L./, carp /Cyprinus carpio L./, bream /Abramis brama L./, and pikeperch /Stizostedion lucioperca L./. It was stated that the heavy metal content of the muscular tissues of the species investigated - apart from one specimen - did not surpass the limit allowed in the majority of the European countries. However, the muscle samples of the Danube barbel contain 8.89 mg/kg Pb instead of the accepted 2.5 mg/kg. This should be explained by the intensive pollution of the Danube. (In addition to Pb, our investigation was extended to the following elements: Hg, Cd, Mn, Cu and Zn, respectively.) Further it was established that the muscular parts serving as food, contain significantly less heavy metal than that allowed for public consumption. In addition, we note that the fish in the Tisza river proved to be minimally contaminated with the heavy metal analysed.

INTRODUCTION

Before any water pollution have started, there have always been heavy metals of geochemical origin /e.g. lead, mercury, copper, etc./ in our surface waters. But these materials could be detected only in traces. Since some decades this favourable

situation has changed all over the world and in our country, too. Primarily the increased industrial production, the road traffic, to a smaller extent, the pollution of agricultural and communal origin increased the heavy metal content both on land and in water, as well as in the top organism of the water, i.e. in the fish. Since one part of heavy metals /e.g. lead, mercury, cadmium/ is distinctly dangerous and toxic, their detection and control is very important and topical from the viewpoint of both water protection and public health.

Heavy metals accumulating in aquatic animals are usually measured in most countries, which are evaluated as compared to the national limits.

Many investigators have dealt and are dealing with the detection of mercury in the water and in fishes, among others, Gergely et al. /1977/, Horváth et al. /1975/, Lévay /1975/, Lukjanienko /1974/, Meyer /1972/, Müller /1981/, Wachs /1982/.

According to Irukuyama /1966/ and Schäperclaus /1979/, heavy metal accumulates primarily in the liver and, to a smaller extent, in the muscle. According to the standards of most countries as also of Hungary, the upper limit of mercury is 0.5 mg/kg body weight. Gergely et al. /1977/ found a quantity of 0.3 mg/kg in the muscle of fishes in Lake Balaton. Salánki et al. /1981/ analysed Chironomus larvae, crayfish of lower-classes, shell-fish and fish for the content of mercury, cadmium, lead, copper, iron, manganese and zinc. During these studies they have established - among others - that mercury was present in a quality of 1.88 mg/kg body weight in crayfish of lower-classes.

Lead can accumulate primarily in the bones and the connective tissues. Home standard permits 2.5 mg/kg of this element. According to Reichenbach-Klinke /1974/, the lead content of fish living in the Rhine varies between 0.5-6.0 mg/kg body weight, this value is higher than the limit of tolerance.

Cadmium can accumulate primarily in the kidneys and testis and can cause disturbances, e.g. atrophy, increased blood pressure, etc. As far as the limit of tolerance is concerned, it is very low, it can only be 0.3 mg/kg. Unfortunately, there are waters polluted to such an extent where, according to

Reichenbach-Klinke, the quantity of cadmium is higher than 5-100 mg/kg body weight in fish.

In our own experiments, between 1979-1982 we analysed the mercury, lead, cadmium, manganese, copper and zinc contents of the liver and muscle tissues of fishes from Lake Balaton, the Danube and the Tisza.

The aim of our studies was to determine to what extent the abovementioned heavy metals occur in the liver and muscle tissues of the fish living in our natural waters. We investigated also how the values determined by us, differed from the quantitative data determined by the home standards. All these have particular importance from the point of view of fish health and human health.

MATERIAL AND METHODS, COLLECTING SITES

Ten specimens of each fish species were examined at least /Tables I-XII show the exact numbers/. With each sample the spot and date of sampling and the length and weight of the fish are shown. The liver and muscle samples were analysed separately.

Preparation of the samples for mercury testing was made according to the OÉTI's Book of Methods in a closed disintegrator. About 5 g of liver and muscle tissue were used. Ten ml of a mixture of nitric acid and sulphuric acid /2:1/ and hydrogen peroxide /10-20 ml/ were used. The prepared samples were stored in polyethylene vessels in a display-type food freezer at -20 ^{o}C up to the atomic absorption measurement.

For the analysis of cadmium, manganese, copper, lead and zinc tissue samples of about 15 g were stored in Kjeldahl flasks of 100 ml volume with 10 ml of mixture of nitric and sulphuric acids /2:1/ overnight (prefacturing), thereafter the organic matter was completely destructed with 20-30 ml of hydrogen peroxide. The surplus oxidizing solvent was boiled out with 5 ml bidistilled water, the residuum was filled up to 10 ml with bidistilled water, filtered through metal-free

filter-paper and stored in glass vessels at -20 °C up to the atomic absorption measurement.

Table I. Spot and date of sampling: Danube, Budapest, November 22, 1979. Fish species examined: Barbel, 10 three- and four-summer-old specimens

	Hg	Pb	Cd	Mn	Cu	Zn
	MUSCLE TISSUE (mg/kg wet weight)					
Mean	0.113	8.89	--	2.35	0.90	9.67
± SD	0.086	2.48	--	0.68	0.32	5.09
	LIVER TISSUE (mg/kg wet weight)					
Mean	0.145	3.51	--	2.08	2.90	6.81
± SD	0.199	3.59	--	3.18	1.67	4.77

Note: Metal designated by + was not tested

Table II. Spot and date of sampling: Danube, Ercsi, October 15, 1980. Fish species examined: Barbel, 10 three- and four-summer-old specimens

	Hg+	Pb+	Cd	Mn+	Cu+	Zn+
	MUSCLE TISSUE (mg/kg wet weight)					
Mean	--	--	0.24	--	--	--
	LIVER TISSUE (mg/kg wet weight)					
Mean	--	--	0.54	--	--	--

Note: Metal designated by + was not tested

Table III. Spot and date of sampling: Balaton Siofok, December 11, 1980. Fish species examined: bream, 10 three- and four-summer-old specimens

	Hg+	Pb+	Cd	Mn+	Cu+	Zn+
	MUSCLE TISSUE (mg/kg wet weight)					
Mean	--	--	0.07	--	--	--
	LIVER TISSUE (mg/kg wet weight)					
Mean	--	--	0.12	--	--	--

Note: Metal designated by + was not tested

Table IV. Spot and data of sampling: Balaton, Siofok, November 27, 1981. Fish species examined: pike-perch, 10 three- and four-summer-old specimens

	Hg	Pb	Cd	Mn	Cu	Zn
	MUSCLE TISSUE (mg/kg wet weight)					
Mean	0.04	1.30	0.19	0.43	0.23	6.80
± SD	0.01	0.43	0.09	0.13	0.10	0.77
	LIVER TISSUE (mg/kg wet weight)					
Mean	0.02	0.57	0.08	1.02	1.19	16.49
± SD	0.01	0.45	--	0.29	0.14	2.77

Table V. Spot and date of sampling: Balaton, Keszthely, November 28, 1981. Fish species examined: pike-perch, 10 three-summer-old specimens

	Hg	Pb	Cd	Mn	Cu	Zn
	MUSCLE TISSUE (mg/kg wet weight)					
Mean	0.03	2.02	0.29	0.53	0.29	0.52
± SD	0.01	0.76	0.15	0.18	0.10	3.67
	LIVER TISSUE (mg/kg wet weight)					
Mean	0.01	2.51	0.12	0.99	2.52	18.84
± SD	0.01	1.21	--	0.10	1.12	3.51

Table VI. Spot and data of sampling: Balaton, Keszthely, November 28, 1981. Fish species examined: bream, 20 three-summer-old specimens

	Hg	Pb	Cd	Mn	Cu	Zn
	MUSCLE TISSUE (mg/kg wet weight)					
Mean	0.10	1.07	0.23	1.95	0.35	13.50
± SD	0.10	0.87	0.09	0.26	0.07	2.43
	LIVER TISSUE (mg/kg wet weight)					
Mean	0.10	1.70	0.25	2.13	4.86	34.93
± SD	0.10	1.32	0.19	1.27	2.26	5.38

Table VII. Spot and date of sampling: Balaton, Balatonszemes, November 16, 1982. Fish species examined: pike-perch, 10 three- and four-summer-old specimens

	Hg	Pb	Cd	Mn	Cu	Zn
	MUSCLE TISSUE (mg/kg wet weight)					
Mean	0.15	--	--	0.04	0.09	5.64
± SD	0.20	--	--	0.01	0.03	1.48
	LIVER TISSUE (mg/kg wet weight)					
Mean	0.15	0.15	--	0.36	1.02	17.52

Table VIII. Spot and date of sampling: Balatonszemes, November 16, 1982. Fish species examined: bream, 10 three- and four-summer-old specimens

	Hg	Pb	Cd	Mn	Cu	Zn
	MUSCLE TISSUE (mg/kg wet weight)					
Mean	0.5	0.16	--	0.10	0.15	5.00
± SD	0.1	0.05	--	0.03	0.11	0.75
	LIVER TISSUE (mg/kg wet weight)					
Mean	0.5	0.20	--	0.47	4.75	19.37

Table IX. Spot and date of sampling: Balaton, Keszthely, November 16, 1982. Fish species examined: pike-perch, 10 three- four- and six-summer-old specimens

	Hg	Pb	Cd	Mn	Cu	Zn
	MUSCLE TISSUE (mg/kg wet weight)					
Mean	0.04	--	--	0.06	0.13	4.44
± SD	0.03	--	--	0.01	0.04	0.61
	LIVER TISSUE (mg/kg wet weight)					
Mean	0.02	0.14	0.07	0.39	1.02	16.36
± SD	0.01	0.03	--	0.07	0.14	1.70

Table X. Spot and date of sampling: Balaton, Keszthely, November 16, 1982. Fish species examined: carp, 10 four- and five-summer-old specimens

	Hg	Pb	Cd	Mn	Cu	Zn
	MUSCLE TISSUE (mg/kg wet weight)					
Mean	--	--	--	0.07	0.49	11.40
± SD	--	--	--	0.02	0.32	4.76
	LIVER TISSUE (mg/kg wet weight)					
Mean	--	0.14	--	0.28	4.15	71.08
± SD	--	0.04	--	0.04	3.15	20.30

Table XI. Spot and date of sampling: Tisza, Szeged, October 7, 1982. Fish species examined: barbel, 10 three- and four-summer-old specimens

	Hg	Pb	Cd	Mn	Cu	Zn
	MUSCLE TISSUE (mg/kg wet weight)					
Mean	0.1	0.31	--	0.12	0.08	4.08
± SD	0.5	0.08	--	0.05	0.05	0.71
	LIVER TISSUE (mg/kg wet weight)					
Mean	--	0.46	0.11	0.41	4.89	11.27
± SD	--	0.12	0.07	0.13	4.90	3.00

Table XII. Spot and date of sampling: Tisza, Szeged, October 7, 1982. Fish species examined: bream, 10 three-, four- and five-summer-old specimens

	Hg	Pb	Cd	Mn	Cu	Zn
	MUSCLE TISSUE (mg/kg wet weight)					
Mean	0.05	--	--	0.66	0.37	7.43
± SD	0.03	--	--	0.26	0.22	2.45
	LIVER TISSUE (mg/kg wet weight)					
Mean	0.01	0.27	0.69	1.21	8.65	31.03

Table XIII. Conditions of atomic absorption measurements

Characteristics	Copper	Manganese	Zinc	Cadmium	Lead	Mercury
Type of instrument	Zeiss	Zeiss	Zeiss	Zeiss	Zeiss	Perkin Elmer
Current of lamp /mA/	6.0	7.0	10.0	5.4	3.0	5
Wave-length /mm/	324.8	279.5	213.0	228.7	283.1	254.2
Gap width /mm/	3.0	3.0	3.5	3.0	4.0	0.7
Method of measurement	by flame	by flame	by flame	by flame	by flame	by quartz cuv.
Combustible gas: acetylene l/h	82	80	82	82	90	---
Gas supplying combustion: air, l/h	480	480	480	480	480	---
Rinsing gas: nitrogen, bar	---	---	---	---	---	3
Lengthening of scale, %	80-100	90-100	0-100	50-100	90-100	---

Table XIV. Concentrations of the calibrating working solutions

Metal tested	1	2	3	4	5	6	7
Copper	0.25	0.50	0.75	1.00	1.25	1.50	1.75
Manganese	0.05	0.10	0.25	0.50	0.75	1.00	1.50
Zinc	0.20	0.40	0.60	0.80	1.00	1.50	---
Cadmium	0.05	0.10	0.20	0.50	0.75	1.00	---
Lead	0.05	0.10	0.20	0.50	0.75	1.00	1.50
Mercury	0.005	0.01	0.02	0.03	0.04	---	---

Conditions of the atomic absorption analysis are shown in Table XIII.

Analysis of mercury was performed by Perkin Elmer attachment type MHS-10. For evaluation 10-20 ml of the sample solutions were measured into the storing vessels. Some drops of potassium permanganate and 0.5 ml of antifoaming agent (n-oktyl alcohol) were added to the samples. Mercury was desoxidized by sodium borohydride of 3% dissolved in 1% sodium hydroxide and was put with the aid of nitrogen into the quartz cuvette placed in the direction of light.

Working solutions for calibration were diluted from the Carlo Erba stock-solution of 1 mg/ml. Concentrations of the working solutions are shown in Table XIV.

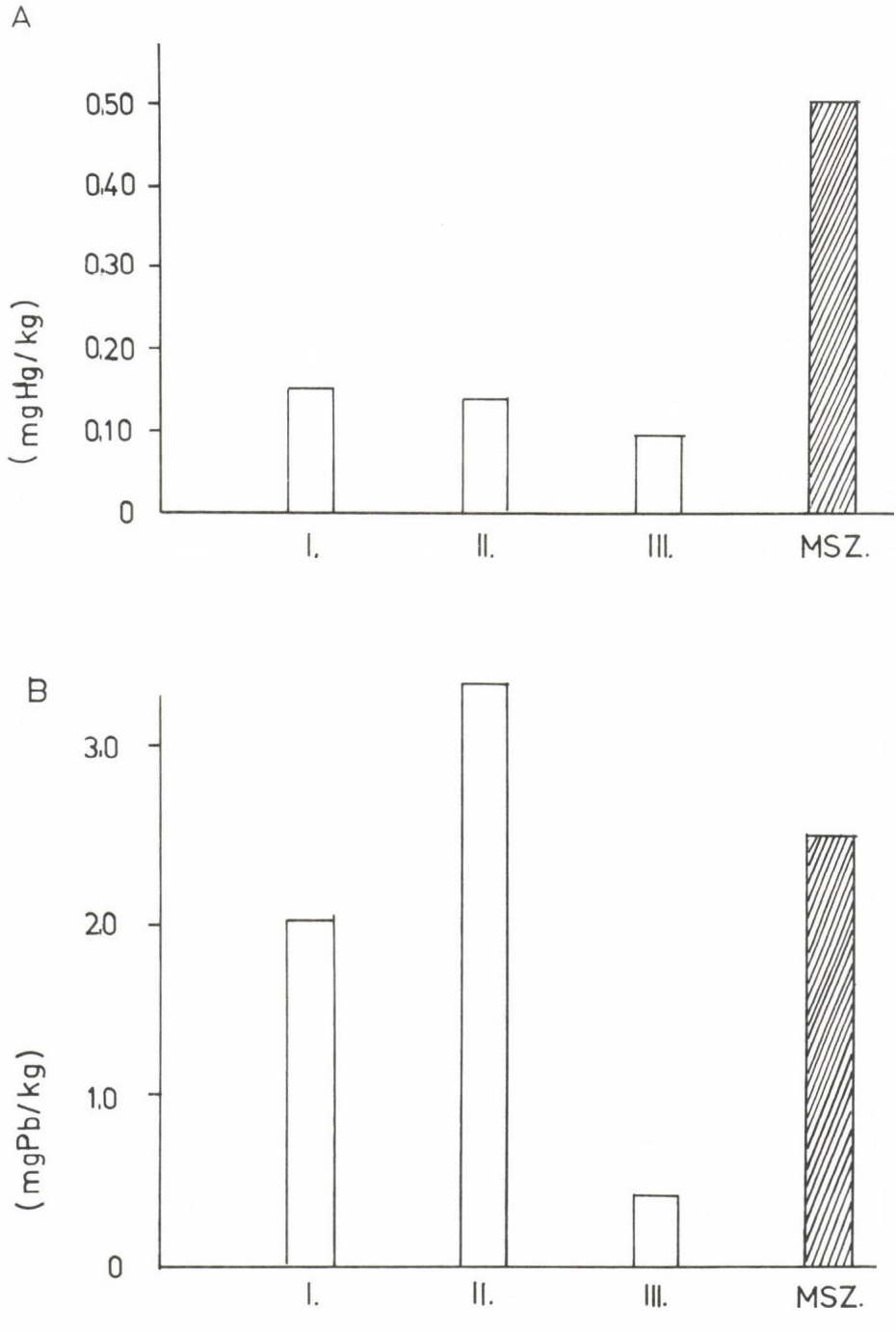
Dry matter content of the samples was determined following 24 hours drying at 105 ^{o}C.

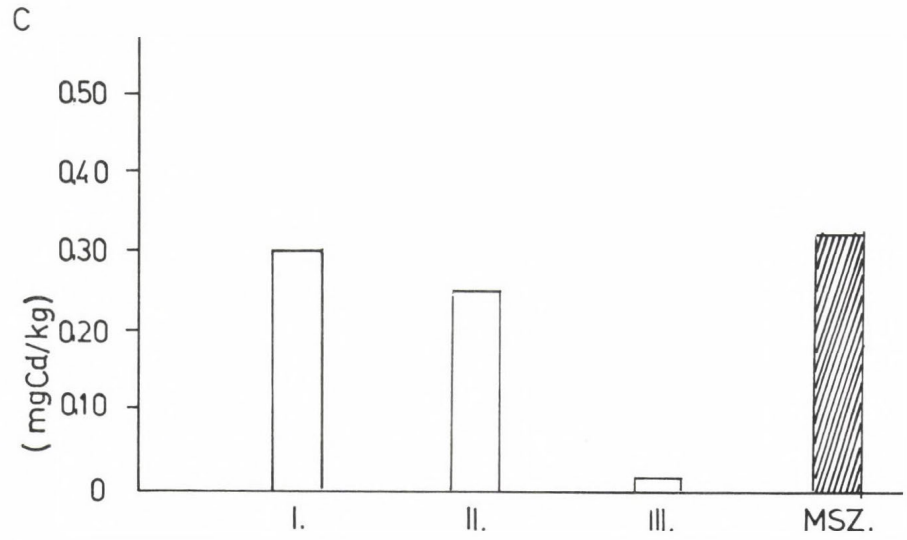
RESULTS AND CONCLUSIONS

Loading levels of heavy metal of some characteristic fish species of Lake Balaton, the Danube and the Tisza - barbel, carp, bream and pike-perch-are shown in Tables I-XII. The heavy metal contents of the muscle and liver tissues were analysed separately. The values are shown in the abovementioned Tables.

We have concluded that - except for one sample, the muscle tissues of fish examined did not contain more heavy metal than permitted by the home standards.

Out of the metals the Order No. 4 of the Ministry of Health /1978/ /25.6/ permits the following limits: mercury 0.5 mg/kg-, lead 2.5 mg/kg-, cadmium 0.3 mg/kg-, manganese 7.5 mg/kg- (= this limit is not standardized but it is the natural content according to Reichenbach-Klinke /1974/), copper 10.0 mg/kg-, zinc 50.0 mg/kg body weight. The only exception was the case of barbel in the Danube, the muscle tissue of which contained 8.89 mg/kg body weight of lead instead of 2.5 mg/kg allowed by the limit. The high lead content of barbels indicates the heavy pollution of the Danube.


A
0.50
0.40
0.30
0.20
0.10
0
(mgHg/kg)
I.
II.
III.
MSZ.
B
3.0
2.0
1.0
0
(mgPb/kg)
I.
II.
III.
MSZ.


C
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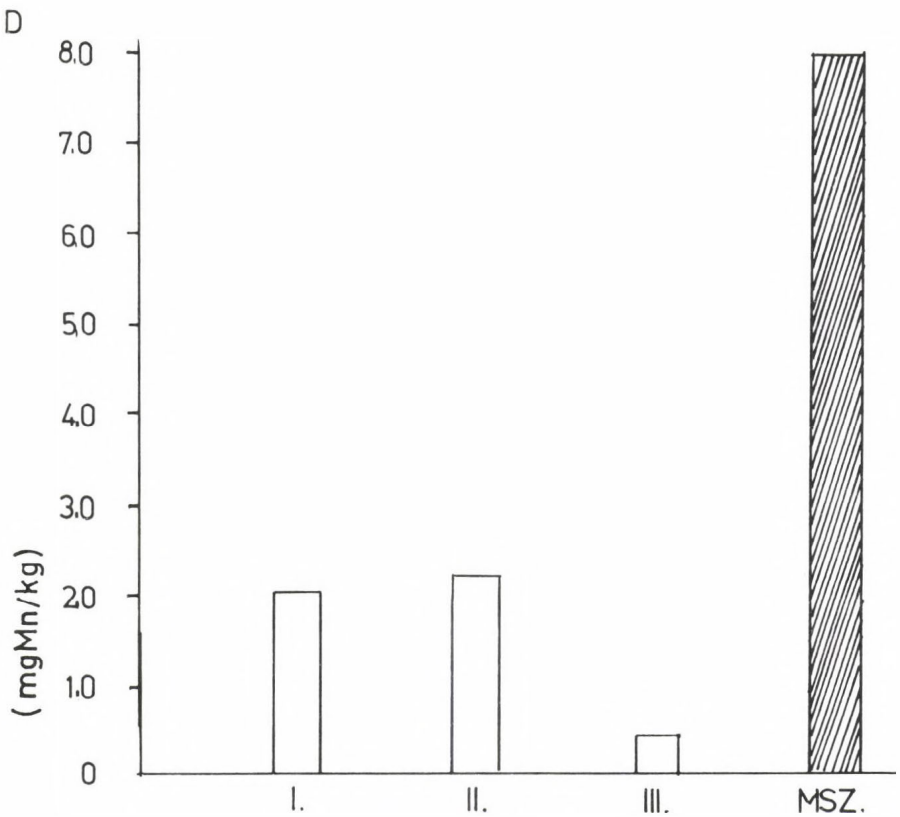
0.40
0.30
0.20
0.10
0
(mgCd/kg)
I.
II.
III.
MSZ.


D
8.0
7.0
6.0
5.0
4.0
3.0
2.0
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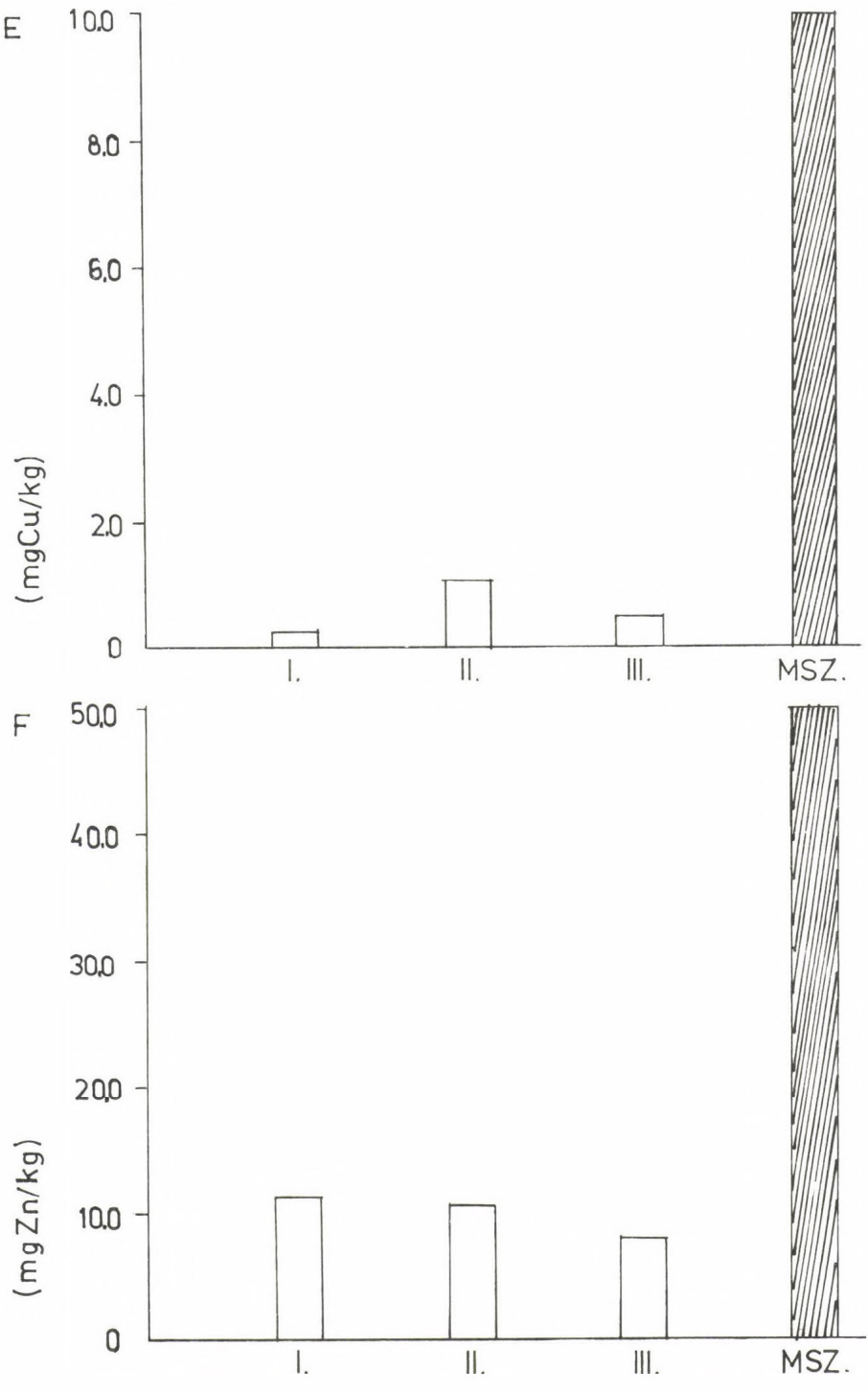
0
(mgMn/kg)
I.
II.
III.
MSZ.


E
10.0
8.0
6.0
4.0
2.0
0
(mgCu/kg)
I.
II.
III.
MSZ.
F
50.0
40.0
30.0
20.0
10.0
0
(mgZn/kg)
I.
II.
III.
MSZ.

We have also established that the muscle tissue, i.e. the part consumed, contained essentially less heavy metals, than the liver tissue not consumed. Noteworthy is the phenomenon that out of the three waters tested, the fish in the Tisza were the least loaded with heavy metals. See Figs A,B,C,D,E,F where the maximum loading levels are shown together with the referring standards (explanation of Figures: I=Balaton, II=Danube, III= Tisza, MSZ=Hungarian Sandard).

SUMMARY

A study was performed in 1979-1982 concerning the heavy metal content of some characteristic fish species of Lake Balaton, the Danube- and the Tisza river.

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ACCUMULATION OF HEAVY NETALS IN AQUATIC ORGANISMS OF SEWAGE WATER TREATMENT PLANTS

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ABSTRACT

The heavy metal concentrations of some aquatic organisms living in two BMKO-type /bio-mechanical combined oxidation system, constructed of several concentric landbasins/ were investigated. Zinc, copper, iron and molybdenum concentrations were determined by atomic absorption spectrometry, Moina rectirostris, Daphnia magna, Sigara lateralis, Chironomus annularius and Oligochaeta were chosen as typical organisms of these systems. Measure of heavy metal accumulations in progress of the purification process was studied. It demonstrated a strong decreasing tendency during the purification in the organism. Among the organisms investigated Moina rectirostris could be characterized by the highest heavy metal concentrations.

INSTRODUCTION

Heavy metals are considered as the most dangerous inorganic micro-pollutants presently. Since these elements can be accumulated in living organisms and can be transferred by the food-chain,they may be dangerous for man.

When we intend to discover the measure of pollution in water, we may analyse the heavy metal content of the waterspace, sediment and aquatic organisms. Though the mediatory substance is water, in most cases investigations of heavy metal accumulation in living organisms /Fjerdingstad et. al.. 1975; Foster, 1982a,b; Newman and McIntosh, 1982; Klumpp and Burdenjones, 1982/ have been favoured recently, because certain

organisms can respond to heavy metal pollution very sensitively.

In this paper heavy metal accumulations of several aquatic organisms living in sewage treatment plants are described. Through our studies we paid attention to investigate these organisms at different steps of purification.

MATERIALS AND METHODS

Our investigations were carried out on the BMKO-type sewage treatment plants of Hajdúböszörmény and Püspökladány in summer 1982.

The bio-mechanical combined oxidation /BMKO/ sewage treatment system is a combined version of a series of connected stabilizing landbasins installed in one structure and surrounded by banks where, according to the number of stabilizing basins sewage flows through a five- or nine-step biological purification process in the traditional sense /Dévai, 1977a,b; Dévai and Woynarovich, 1981/. The untreated or preliminarily settled sewage gets into the central basin from where it flows through either four or eight further basins as required by a shunt-connected or a cascade-connected arrangement.

In Hajdúböszörmény the sewage mainly comes from households but a large quantity /about 50 %/ of industrial sewage is loaded on the BMKO-system at present. Among the industrial plants having sewage effluents the most important one is an electronics factory. In 1979 controlling its effluents, we already took note of very high /200-300 mg litre^{-1}/ molybdenum concentration of sewage. In 1982 due to our efforts this contamination was stopped.

Contrasted with Hajdúböszörmény, in Püspökladány nearly all of the sewage load on the BMKO-system is communal. There are not any industrial effluents presently.

In order to trace the different steps of purification we chose three basins of BMKO-systems. Thus we took samples from typical organisms /Cladocera, Crinomida larvae and water bugs/ of basin B, D and K.

Among the organism samples zooplankton was collected by plankton net /typ. 25-I/A/ at some points of the basins. The

zooplankton samples were cooled and carried in clean, glass bottles to the laboratory, where sorting and identification of species were completed. Sigara lateralis was separated from zooplankton samples and it was determined.

Sediment-dwelling organisms of sewage treatment plants were collected from samples taken by Hargrave-sampler /Hargrave, 1969/. The samples were carefully washed through a 0.8 mesh sieve. The animals were picked out from the residue using Leonhard-tweezers then put into clean plastic boxes and carried to the laboratory in a cooling bag.

For determination of heavy metal contents, suitable amounts of organisms were treated in teflonbomb with 5 ml cc. HNO_3 and 3 ml cc. H_2O_2 at 150 ^{o}C for 3 hours. Of course, the dry matter contents of organisms were determined after drying at 105 ^{o}C.

Measurements were carried out using a Perkin-Elmer Model 5000 Atomic Absorption Spectrophotometer having two light-beams, Czerny-Turner type optics controlled by microprocessor and an automatic burner control unit. Both flame and electro-thermal atomization /HGA-500 Graphite Furnace/ techniques were applied /Analytical methods etc., 1976/.

For the preparations and measurements Merck, "zur Analyse" reagents were used. Standard solutions were made by diluting 1000 mg litre^{-1} stock solution. All results for organisms are given in terms of mg kg^{-1} dry matter further on.

RESULT AND DISCUSSION

On the basis of the results of our investigations heavy metal accumulations of the aquatic organisms have proved to be very different in the two BMKO-systems.

Zinc accumulation in organisms /cf. Table 1/ is fairly significant in the BMKO-system of Hajdúböszörmény, the organisms investigated can be characterized by zinc concentrations between 169 and 5971 mg kg^{-1}. The highest values were determined in Moina rectirostris living in the basin B. Zinc concentrations of similar magnitude were found in Sigara lateralis. When comparing these values to zinc concentration of Chironomus annularius /about 200 mg kg^{-1}/, we can see that

the bottom-dwelling larvae have a very low zinc accumulation. During the progress of purification zinc loading of organisms decreases. Organisms of the BMKO-system of Püspökladány have a lower zinc accumulation than the organisms of the other BMKO-system. Higher zinc concentrations can also be determined in organisms living in the water than in bottom-dwelling ones.

Table 1
Heavy metal contents in aquatic organisms of BMKO-type sewage treatment plants

Sample	mg kg^{-1} dry matter			
	Zinc	Copper	Iron	Molybdenum
BMKO of Hajdúböszörmény				
Moina rectirostris /B/	5971	156	9716	605
Moina rectirostris /D/	890	6.8	982	27,3
Sigara lateralis /D/	2318	36.5	892	30.9
Sigara lateralis /K/	1552	21.2	767	9,7
Chir. annularius /D/	210	7.6	556	17,0
Chir. annularius /K/	169	4,0	313	4.5
Oligochaeta /B/	887	6.7	2143	60.3
BMKO of Püspökladány				
Daphnia magna /D/	336	8.6	1068	1,2
Daphnia magna /K/	694	2,2	6570	2.2
Sigara lateralis /D/	918	21.0	967	1.3
Sigara lateralis /K/	518	9.2	467	0.0
Chir. annularius /B/	417	16.1	1439	0,0
Chir. annularius /D/	304	8.2	1427	0.0
Chir. annularius /K/	678	11.2	2487	0.0

Copper concentrations have the same characteristics as the quality relations of zinc. Copper accumulation of organisms varies between 2.2 and 36.5 mg kg^{-1} dry matter, except Moina rectirostris living in basin B of the BMKO-system of Hajdúböszörmény. Copper concentrations in organisms, as we also observed in the case of zinc, decreased in progress of

purification.

Iron concentrations in organisms are high /316-9716 mg kg^{-1}/ related to other heavy metal contents. The greatest iron accumulation can be found in Moina rectirostris living in basin B of BMKO-system of Hajdúböszörmény.

The Oligochaeta living in this basin also have an iron concentration of 10^3 mg kg^{-1} magnitude. It is to be noted that iron concentrations in the organisms also decrease in progress of the purification. Amounts of iron in organisms of the BMKO-system of Püspökladány are similar to ones of Hajdúböszörmény except Chironomus annularius, that have values of 1427-2487 mg kg^{-1}.

A shocking case of industrial pollution is shown by molybdenum concentrations of organisms living in the BMKO of Hajdúböszörmény. As we mentioned at characterization of this test object, industrial effluent with very high molybdenum concentration polluted the sewage system of Hajdúböszörmény. The fate marks of this pollution can be shown by very high molybdenum contents of organisms living in the BMKO of Hajdúböszörmény related to Püspökladány's ones. Moina rectirostris so accumulated this element in the highest degree /605 mg kg^{-1}.

Though heavy metal concentration of organisms can be very important data for detection of certain pollution, the simultaneous investigations of the waters, sediments, organisms and geochemical background /Förstner and Wittmann, 1979/ can demonstrate the heavy metal loading as sewage treatment plants as natural waters.

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ACCUMULATION AND EFFECT OF HEAVY METALS IN THE FISHES OF LAKE BALATON

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Lake Balaton is one of the most important natural resources of Hungary. It is a centre of interest from the viewpoint of environmental protection, fishing and angling. Its food-fish production is 1200-1400 tons per year, which is about 5 % of the total Hungarian food-fish production; thus, Balaton fishes are important for human consumption, too. Death of Balaton fishes is a public matter; therefore it is indispensable to investigate the different toxic factors associated with the Balaton aquatic environment.

The present research work was started at the Central Veterinary Institute in 1976 and carried out by a team formed of the coworkers of the Fish Health and Toxicological Departments. The essence of the complex investigations is as follows: Fishes originating from the catch were examined for their outer look and physical condition, and subsequently by parasitological, pathoanatomical, histopathological, bacteriological, virological and toxicological methods. From the results of these investigations we can conclude to the health situation of the fishes. A part of our research work is demonstrated in this paper.

MATERIALS AND METHODS

Fishes /common carp, common bream and pike-perch/ from the catch of three different areas of Balaton /Siófok, Fonyód, Keszthely/ were examined. Samples were taken 10 times a year,

usually monthly. On each occasion 3 fishes from each of the three species were taken and processed from each place. So far about 1000 fishes have been examined; 785 of them were from Lake Balaton. Three years old or older fish of the same species, taken from the Tihany Inner Lake, the Velence Lake and 12 fish farms were used as controls/Table 1/. Samples for toxicological analysis were taken from the liver and muscles and, sometimes, from the gills, kidney and intestinal contents. The atomic absorption method was used to determine heavy metal levels in fishes. Initially only copper and zinc were determined, because these were used as active substances of pesticides /fungicides/ in vineyards around Lake Balaton. Since 1982, however, cadmium and lead have also been determined, according to Müller's data about Lake Balaton and River Zala.

Histological sections were made from gill, liver, kidney, intestine, and sometimes, skin. The sections were stained with haematoxylin-eosin, Sudan-III and studied for PAS reaction.

Results of the histopathological examination and toxicological analysis were compared.

We attempted to induce the seasonal fluctuation of Cu and Zn levels in common carp of about 300-400 g body weight, collected from fish farm. Each group contained 10 fish. For three months the fish were fed carp and trout complete food, respectively, in which the Zn and Cu levels were as follows: the carp food contained 18.8 ppm Cu and 57.5 ppm Zn, the trout food 56.9 ppm Cu and 230.0 ppm Zn. At the start of feeding some of the carp were killed and examined as controls. At halfway through this study 5 fish were killed, and 5 fish, until then receiving trout food, were fed carp food again. At the end of the experiment all fish were killed and examined by the methods mentioned above.

Heavy metal residues were evaluated on the basis of values allowed for human consumption in the 4/1978 Ministry of Health order; i.e. in wet weight of organs: Zn 80 ppm, Cu 60 ppm; in muscles: Zn 60 ppm, Cu 10 ppm. The allowed value of Cd is 0.3 ppm and that of Pb is 2.5 ppm in organs and muscle as well.

Table 1 Number and species of fish examined

Place	Common carp	Common bream	Pike-perch
Balaton	225	257	303
Tihany Inner Lake	6	-	-
Velence Lake	17	9	8
Fish farms	112	-	-

RESULTS

On the basis of the results the fishes examined were healthy from the veterinary aspect. Their condition was not bad and they did not seem to be ill. The colour changes observed in the liver of 23-27 % of the fishes were associated with the quantity of fat and glycogen, which substances are inversely related; the discolouration was more expressed in summer and less pronounced in autumn and winter.
These colour changes were considered abnormal when the quantity of glycogen characteristic of species and season decreased, and at the same time diffuse pathological and necrobiotic fatty infiltration occurred. However, only a few fish with such liver were found each year.

The quantity of heavy metals did not reach the toxic level; However, it showed considerable variation between the different organs of the fish. Also, there were differences between the same organs of fishes belonging to different species /Table II/. The Zn content was several times higher than the Cu content. In bream and particularly in carp the residues were higher than in pike-perch. There was no significant difference between the same fish species originating from the three areas. In control fishes collected from different places the heavy metal residues were generally lower than in fishes from Lake Balaton. In the pike-perch, heavy metal residue levels were nearly constant throughout

Table II Heavy metal residues in Balaton fishes
(expressed for wet weight of liver and muscle, in ppm)

			Cu	Zn	Pb	Cd
Common carp	liver	min-max	2,55-35,68	33,00-733,40	0,37-3,16	0,01-0,94
		$\bar{x}$	15,05	235,14	2,06	0,08
		s	13,60	183,50	2,00	0,02
	muscle	min-max	0,17-1,72	5,79-25,87	0,36-0,93	0,02-0,68
		$\bar{x}$	1,44	15,35	0,46	0,03
		s	1,06	14,49	0,25	0,01
Common bream	liver	min-max	8,01-32,58	25,20-36,51	0,16-0,88	0,16-0,37
		$\bar{x}$	7,76	25,57	0,47	0,2
		s	2,28	7,4	0,28	0,05
	muscle	min-max	0,47-2,07	3,51-6,64	0,26-1,99	0,03-0,06
		$\bar{x}$	0,94	4,54	0,54	0,04
		s	0,66	0,53	0,33	0,01
Pike-perch	liver	min-max	1,77-9,70	13,12-33,99	0,39-9,96	0,06-0,09
		$\bar{x}$	1,36	14,64	2,02	0,08
		s	0,23	1,41	2,60	0,02
	muscle	min-max	0,48-2,27	2,62-4,13	0,19-0,31	0,04-0,07
		$\bar{x}$	0,5	4,04	0,30	0,05
		s	0,15	0,5	0,06	0,03

the year, and the maximum levels generally did not exceed those allowed by the order of the Ministry of Health.

Heavy metal levels showed wide variation in carp and bream. The Zn level of the carp liver frequently /in more than 20 % of the cases/ exceeded that allowed by the order.

There was no correlation between the amount of residues found by toxicological analysis and the histopathological changes. In the common bream less expressed and in the common carp more pronounced seasonal fluctuation has been observed. Zn and Cu residues were higher in winter, spring and early summer, and the levels of heavy metals were lower in summer and autumn. In the intestinal contents of carp maximum 13 ppm of Cu and 178 ppm of Zn were found in winter, while only 4 ppm of Cu and 18 ppm of Zn in summer.

Data of the three-month feeding trial are shown in Table III. The Cu and Zn levels of carp coming from the same fish-pond were very variable. The data obtained at the end of this study gave no unambiguous evidence indicative of the accumulation of heavy metals.

DISCUSSION

From the above it can be seen that Cu, Zn, Pb and Cd pollution of Balaton aquatic environment is not yet high enough to cause chronic toxicosis or to damage the health of fishes by any other way. This statement is proved by the residues found in the pike-perch because accumulation of these heavy metals could not be observed. The wide variation found in the omnivorous carp and bream showed that high quantities of Zn may accumulate in invertebrates and plants in some habitats in the lake, resulting in a significant increase of Zn quantity in carp. This conclusion has been drawn from the analysis of the intestinal contents, which calls the attention to that the seasonal fluctuations are the consequences of feeding. The Zn levels exceeding the limits allowed for human consumption cause significantly greater problems which are worth to pay atten-

Table III Heavy metal levels in carp fed for three months
(in wet weight)

		Liver		Muscle	
		Cu	Zn	Cu	Zn
Carp, control April 27	min-max	6,99-15,56	32,10-156,44	0,55-1,67	4,64-7,03
	$\bar{x}$	12,0	94,75	0,96	5,27
	s	3,79	58,33	0,55	1,23
Carp, control August 2	min-max	18,18-22,97	70,43-90,97	0,75-0,82	3,01-4,64
	$\bar{x}$	20,58	80,70	0,79	3,83
	s	3,39	14,52	0,05	1,15
Carp, at half-time of feeding	min-max	4,03-15,68	23,08-46,89	0,59-1,37	2,60-20,18
	$\bar{x}$	8,31	36,62	0,98	9,26
	s	5,55	11,50	0,32	8,04
Carp, fed mixed food	min-max	15,11-22,03	55,04-132,80	0,72-1,04	3,83-6,04
	$\bar{x}$	18,67	85,25	0,87	4,84
	s	2,77	31,73	0,12	0,86
Carp, fed for three months	min-max	13,63-27,11	65,07.84,40	1,00-1,54	3,56-4,72
	$\bar{x}$	20,80	72,36	1,21	4,53
	s	5,66	7,40	0,24	0,84

tion to. Our results have not confirmed a higher quantity of Cd in fishes which has been supposed on the basis of Müller's data /1981/.

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DISCUSSION

WACHS, B: You have found very high levels of Zn especially in carp livers. How do you explain these high concentrations? Might it be that the Balaton sediments are contaminated with Zn more than usually? If this is not the case, I believe that this accumulation is rather strange.

H.-MIKLOVICS, M: Unfortunately we did not analyse Balaton sediments, but we know from the literature that Zn level of Balaton sediments is not too high. High level of Zn in carp liver is indeed strange. We believe that the reason for this is a small difference between the feeding habit of carp and the bream. We have supposed that Zn accumulates in invertebrates and plants in some habitats in the lake and there is special food only for the carp. We think it would be useful to find these habitats and foods to solve this problem.

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SEASONAL AND LOCAL VARIATION IN THE HEAVY METAL CONCENTRATION IN ANIMALS OF LAKE BALATON

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The ability of some aquatic organisms to take up and store chemical substances occurring in the environment, like heavy metals, is widely recognized. As a result of such a property these plants and animals reflect the available metal concentration of the environment /Pentreath 1973, Phillips 1976a,b/ and can serve as biomonitors in detecting the level of pollution. Among water animals concentrating heavy metals the filter-feeder bivalvia and a number of planktonic organisms are widely studied /Anderson et al 1978, Cajander 1980, Fowler et al 1978, Theede et al 1979/. In our previous investigations we found that freshwater mussels and planktonic crustaceans living in Lake Balaton concentrate heavy metals in a considerable degree. In total animals or in various organs the factor of bioconcentration varied between 1000 - 100.000 as compared to the concentration of these metals in the water /Salánki et al 1982/.

When evaluating heavy metal concentrations measured in living systems and interpreting these results one should take into consideration a number of factors and circumstances, which may contribute to the setting up of the actually obtained values. These are partly outside circumstances, like temperature, salinity, pH, water depth, partly inside factors, like ontogenetic stage, age and size of the animal, cycle of reproduction and so on /Boyden 1974, Hardstedt-Romeo 1980, Phillips 1976a, 1977a, Romeril 1974/. Some of these variables, both

Fig.1 - Map of catchment area of Lake Balaton. Dots mark sampling stations in the Lake

external and internal are summed up in seasonal variations, which can be considered as a compound factor influencing the heavy metal level of the animals.

In the recent work our intention was to measure heavy metal concentrations in the mussel Unio and in the crustacean plankton collected at different regions of Lake Balaton in various seasons of the year. Four toxic metals were determined, namely Hg, Cd, Pb and Cu, using the method of atomic absorption spectrophotometry /Hatch and Ott 1968, Krishnamurty et al 1976, Paus 1972/.

Zooplankton was sampled in spring, in summer and in autumn, using a net having 300 µm mesh size. The samples contained mainly crustacean plankton, namely Cladocera and Copepoda species. Ten places of sampling were selected /Fig.1/ each of them was located at the open water. Five of them were close to the north shore, the other five in the middle line of the Lake.

Mussels were collected five times between April and September at the west part of the Lake, and once at Balatonfüred, close to a sailing boats harbour. In mussels the gills, foot /including the viscera/, adductor muscle and mantle were separately analyzed.

All data are given in mg/kg dry weight, even in case of Hg which was measured from wet tissue. All the other metals were determined after previous drying of the samples.

The concentration of mercury /Fig.2/ varied between 0.236-0.964 mg/kg at the 10 sampling stations. Values were in general higher closer to the shore than in the centre of the Lake and there was a reduction of Hg concentration in samples towards east.

Concentration of cadmium /Fig.3/ varied between 1.26 and 35.0 mg/kg. Higher values were measured in crustaceans collected in the western part of the Lake both close to the shore and to the middle line. Also high value was found at the most eastern point close to the shore.

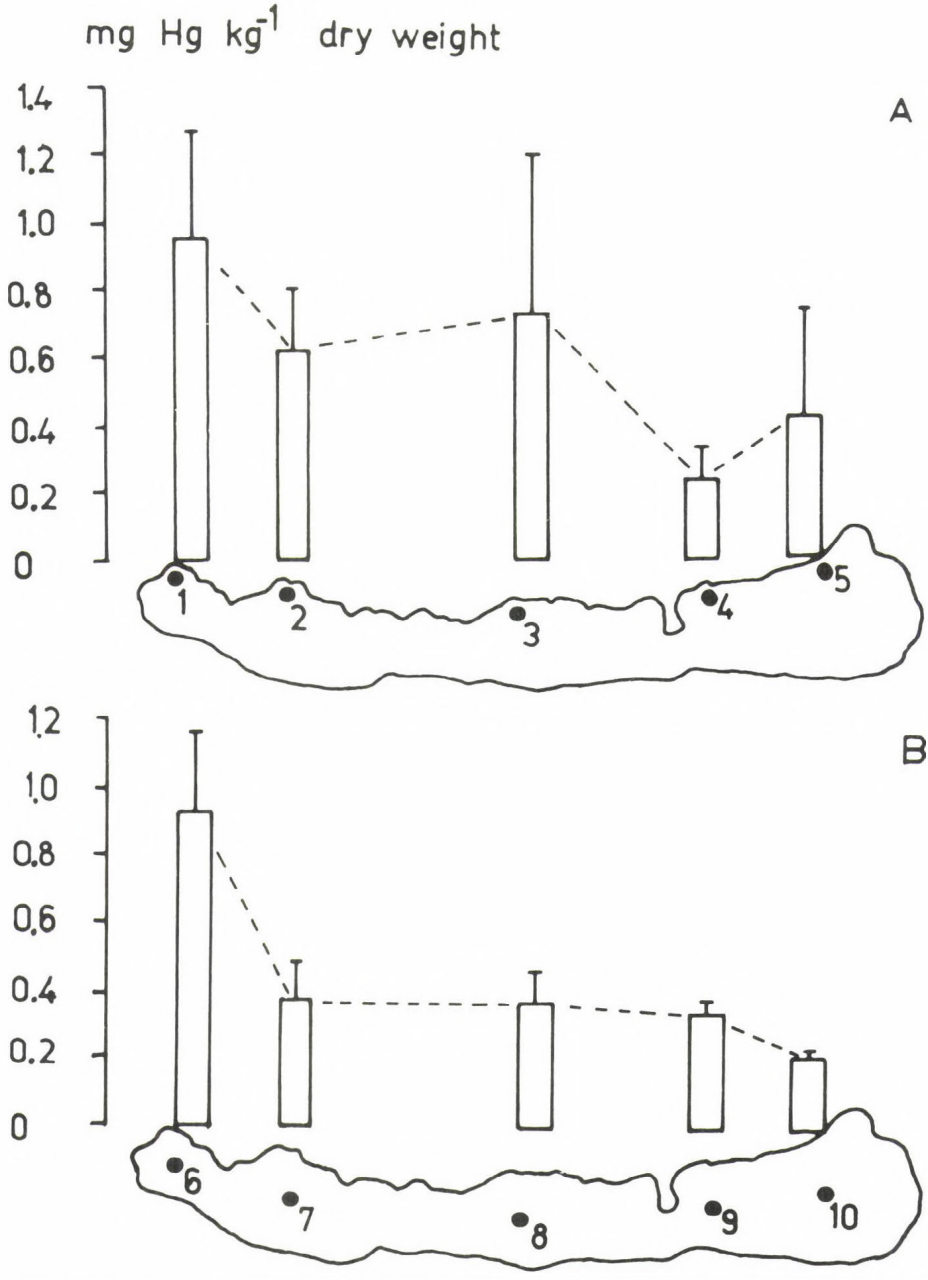

Fig.2 - Mercury concentration in crustacean plankton closer to the north shore /A/, and in the middle line /B/ of Lake Balaton

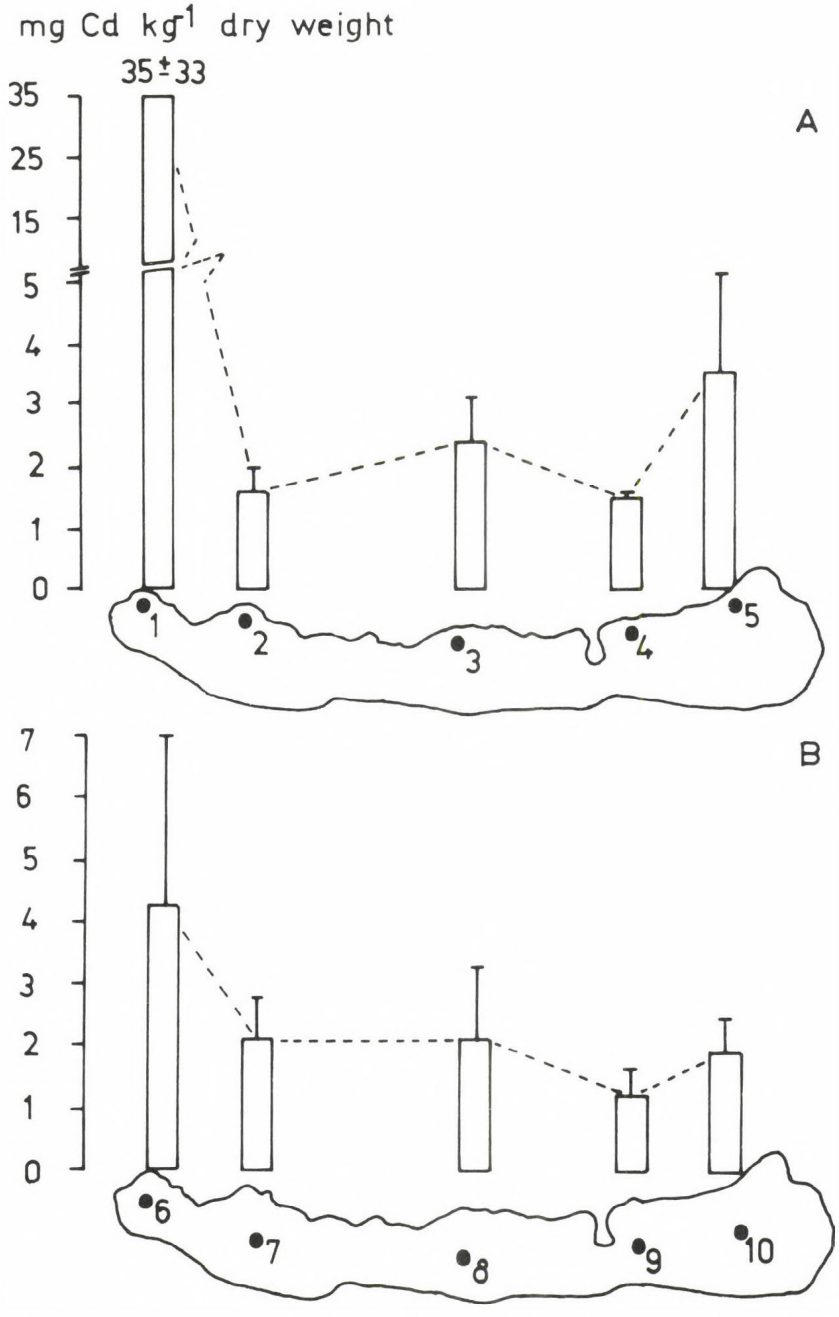

Fig.3 - Cadmium concentration in crustacean plankton closer to the north shore /A/, and in the middle line /B/ of Lake Balaton

Copper concentrations /Fig.4/ were in the interval of 8.38 and 57.3. No significant differences were found in the samples taken in the middle of the Lake, however, high values were measured in samples collected at the western part, close to the shore.

Lead concentration /Fig.5/ varied in the crustacean plankton between 11.6 and 61.0 mg/kg. Highest values were found in the samples taken in the centre of the western basin. The decrease of the lead concentration towards east was not uniform, because at the most eastern point there was an increase again.

There were differences between values of Hg, Cd and Pb concentrations /Fig.6/ of the crustacean plankton collected in three different seasons. Namely, Hg and lead were higher in summer than in autumn, the concentration of cadmium was increasing from spring to summer and further on to autumn. In the concentration of Cu /Fig.6/ no significant differences were found seasonally.

Seasonal sampling was carried out for mussels at the western part of the Lake /Fig.1 - Sampling station No.6/.

It was found that the concentration of Hg and Pb remained at the same level in the gills /Fig.7/ between April and September, while Cu increased by about 10 times and Cd by twice. Contrary to this, the concentration of lead and mercury doubled in the foot /Fig.8/ in summer, and the concentration of copper increased up to September. Except for the April value, practically no differences were found in this organ in the concentration of cadmium.

In the adductor muscles /Fig.9/ no significant differences were found in the lead concentrations between April and September, but copper concentration was three times higher in summer as compared to the April mean value. The mercury and cadmium concentrations decreased in June.

In the mantle /Fig.10/ the mercury concentration was seven times higher in summer as compared to the April value. Some slight variations were detected in different seasons, but no striking changes occurred for either of the other investigated heavy metals.

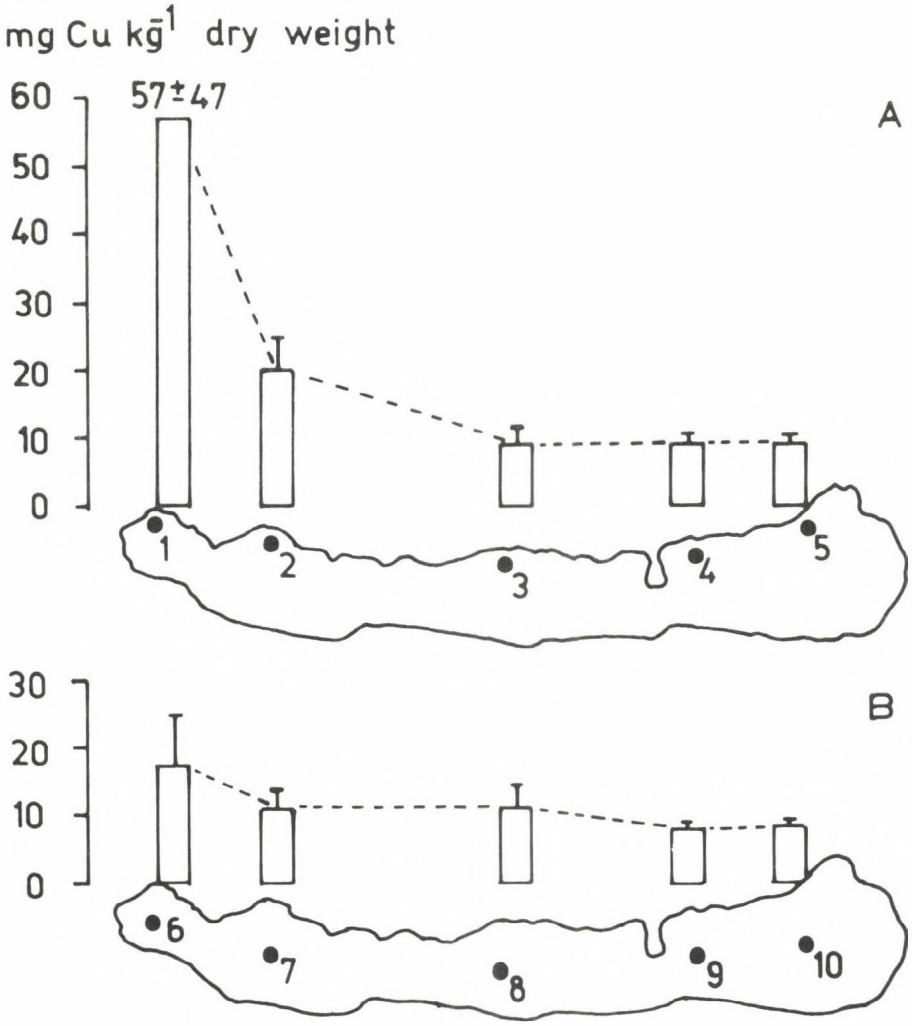

Fig.4 - Copper concentration in crustacean plankton closer to the north shore /A/, and in the middle line /B/ of Lake Balaton

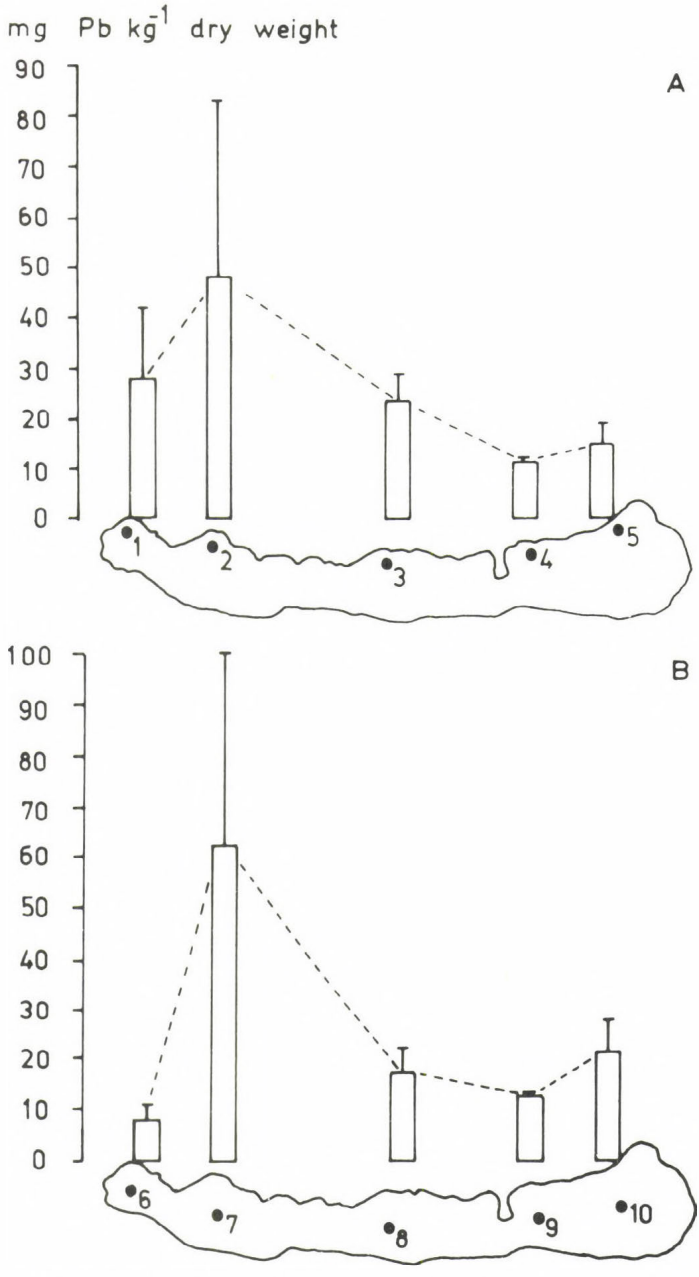

Fig.5 - Lead concentration in crustacean plankton closer to the north shore /A/, and in the middle line /B/ of Lake Balaton

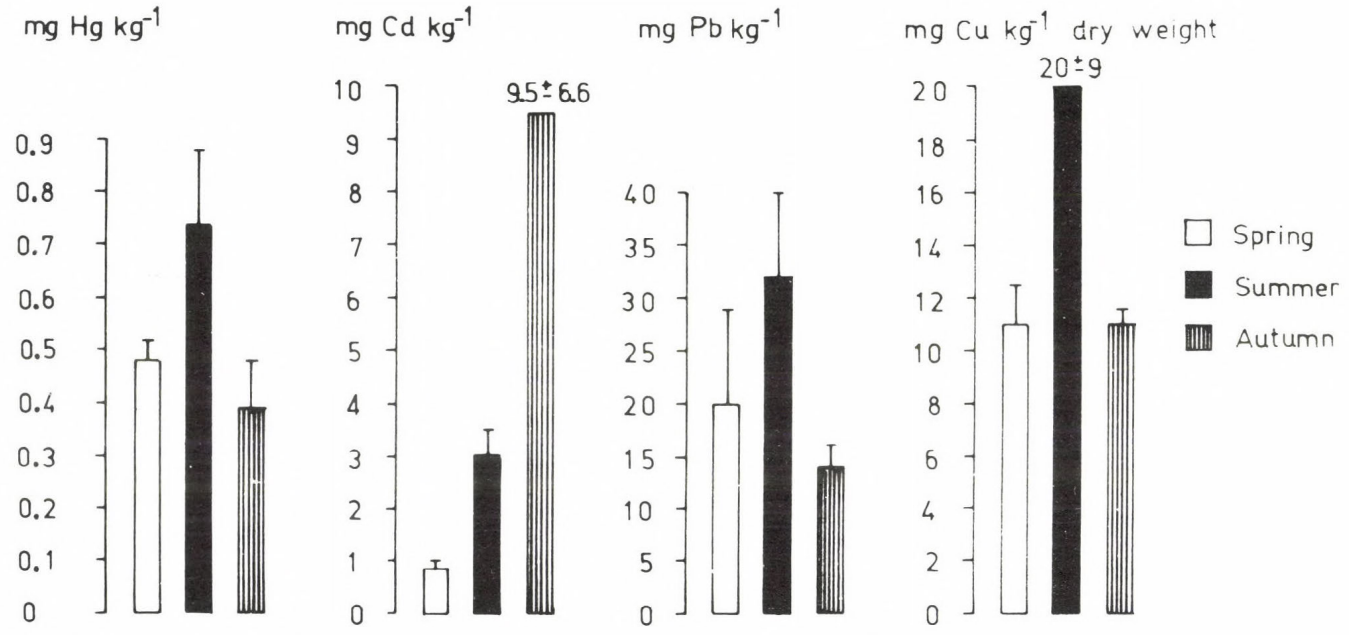

Fig.6 - Mercury, cadmium, lead and copper concentrations in crustacean plankton collected in three different seasons

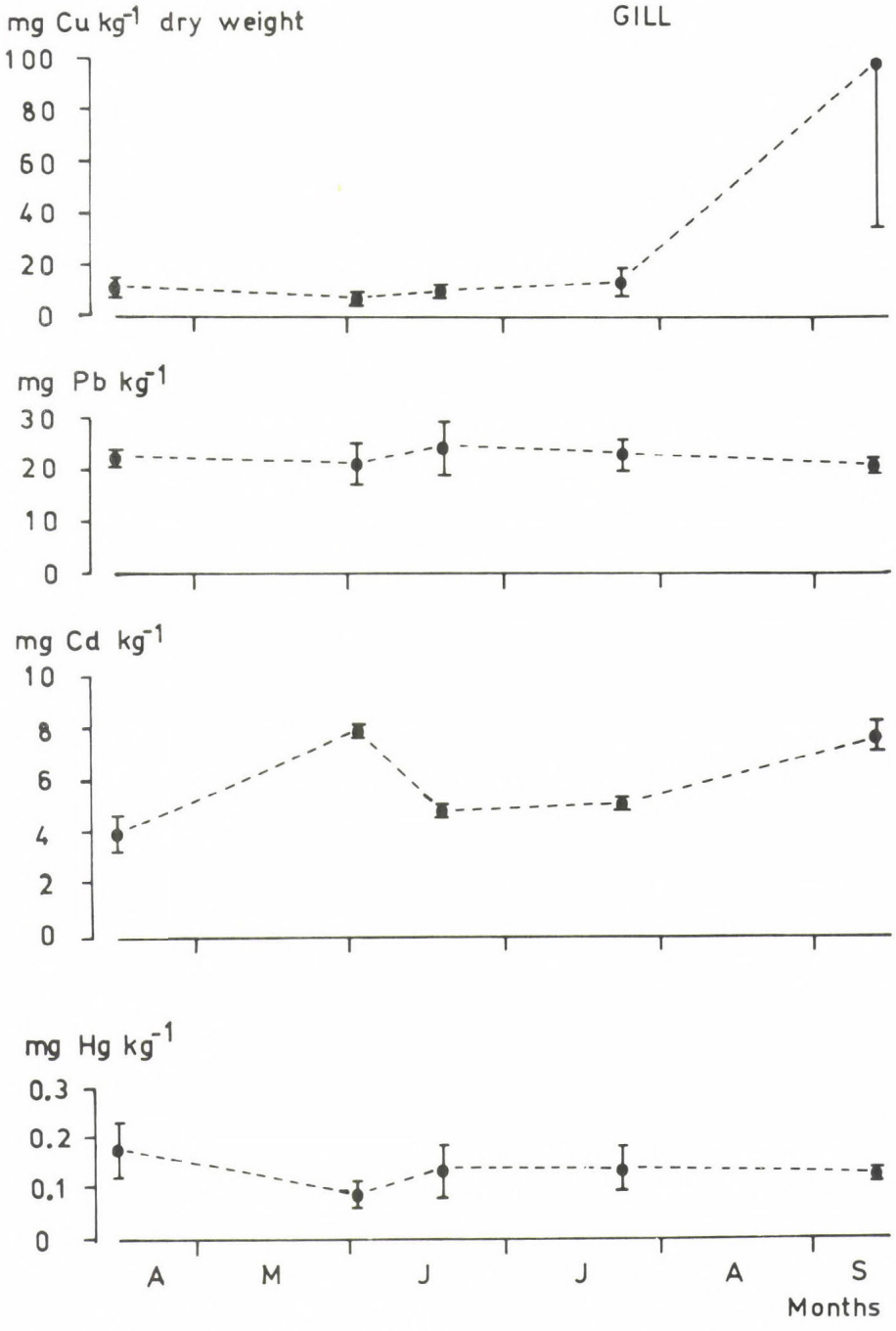

Fig. 7 - Variations of Cu, Pb, Cd and Hg concentrations in the gills of mussels /Unio sp./ collected from the open water of Lake Balaton

The mussels used in the above investigations were collected from the open water of the Lake where they are most numerous. For comparison we took mussels at Balatonfüred too, close to the shore at a place where sailing boats are stored during almost the whole year.

Determination of copper /Fig.11/ showed a very high concentration in the gills, in the adductors and in the mantle as compared to the values measured in mussels collected from the open water of the Lake.

Higher lead values /Fig.12/ were measured in the gills and in the foot, also high values were found in the gills for cadmium /Fig.13/.

In the light of the data published for animals living in various lakes, rivers and seas we can conclude that the heavy metal concentrations of crustaceans and mussels of Lake Balaton are similar to those which were found in animals collected from unpolluted or slightly polluted waters.

Nevertheless, there were significant differences between concentrations of the four investigated metals in various locations of the Lake, referring to the regional differences in heavy metal pollution. The results showing that the zooplankton was more polluted close to the shore than in the middle of the lake clearly refers to the human influence. Also the fact, that in the crustacean plankton there is a reduction of heavy metal concentration from the western part of the Lake towards the eastern one, refers to the influence of the environment. Most of the water from the catchment area arrives to the western part of Lake Balaton with small streams and with the Zala river, bringing a large amount of pollution of agricultural, domestic and partly of industrial origin.

Fertilizers and various chemicals used in agriculture can be responsible for a part of heavy metal pollution, and the heavy traffic around the Lake is responsible for the release of Pb into the environment which in time reaches the Lake and accumulates in aquatic organisms.

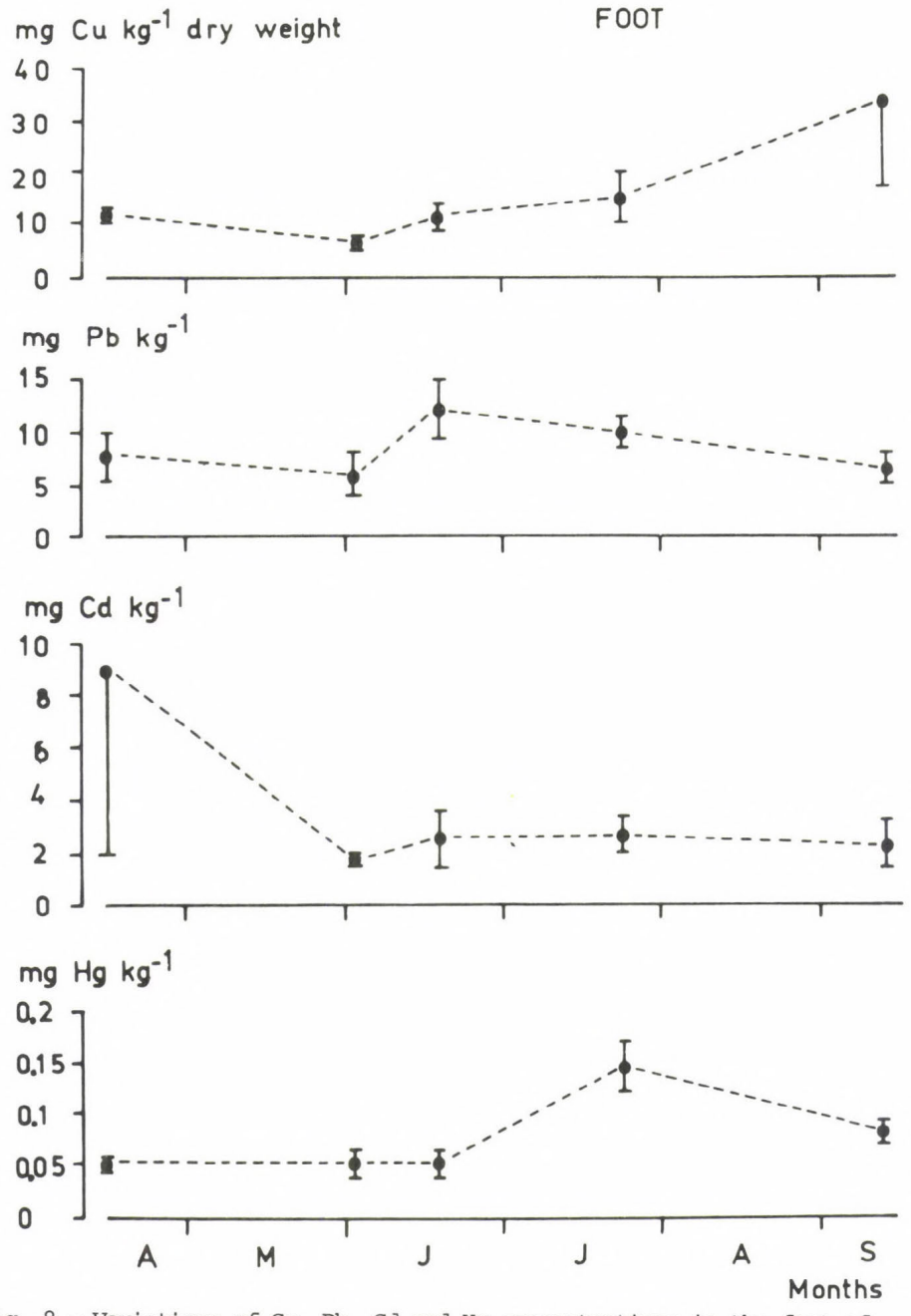

Fig. 8 - Variations of Cu, Pb, Cd and Hg concentrations in the foot of mussels /Unio sp./ collected from the open water of Lake Balaton

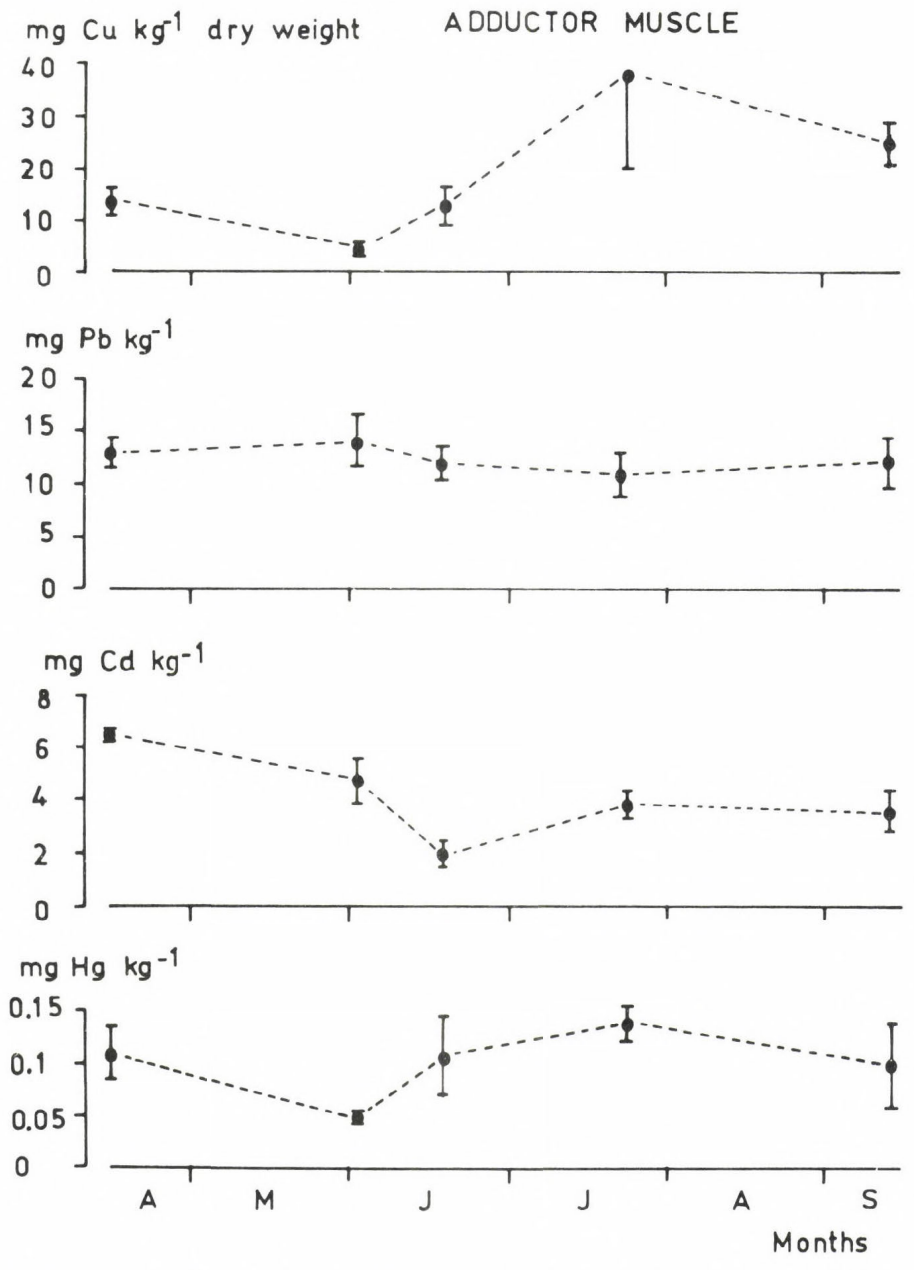

Fig. 9 - Variations of Cu, Pb, Cd and Hg concentrations in the adductor muscle of mussels /Unio sp./ collected from the open water of Lake Balaton

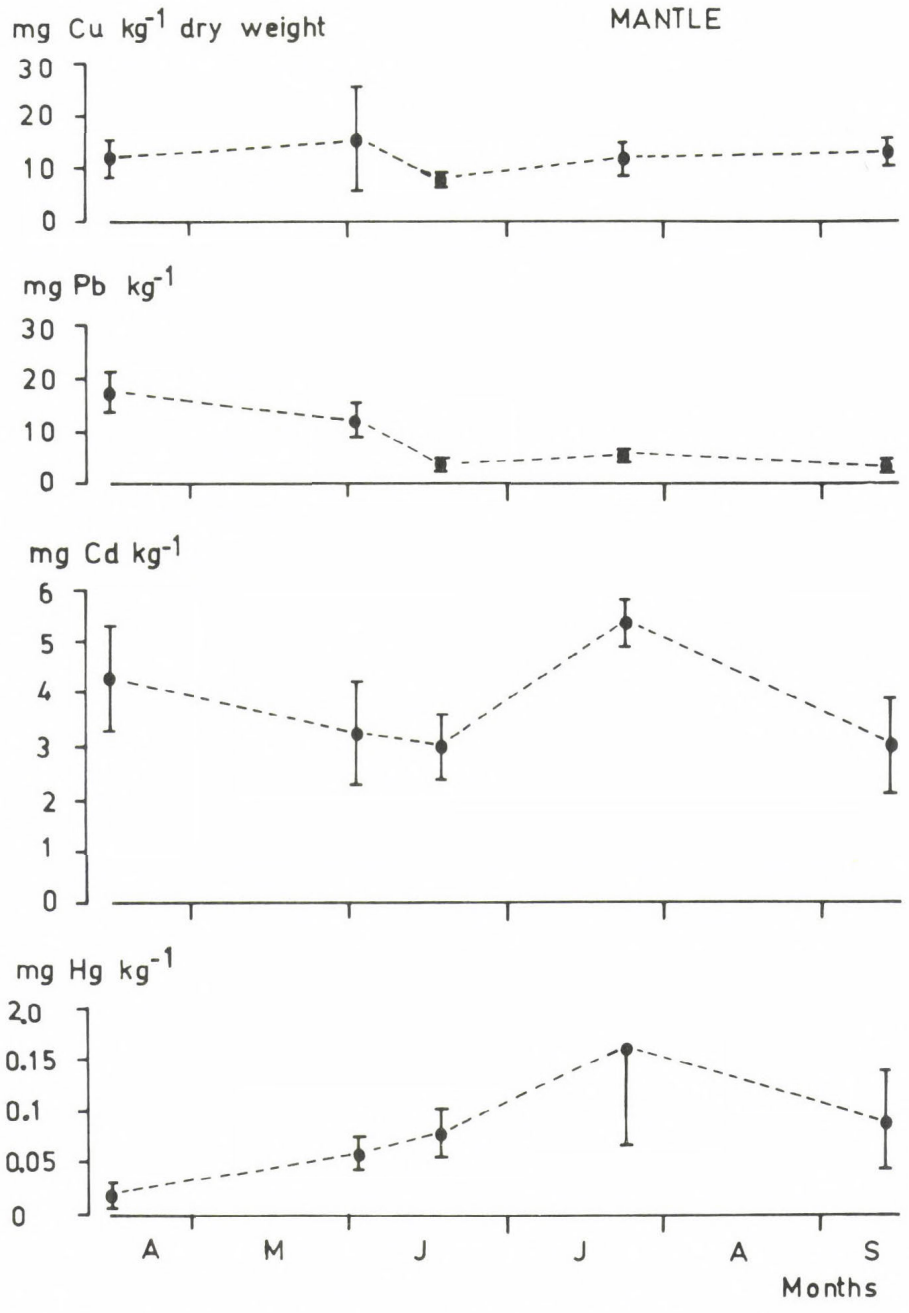

Fig. 10 - Variations of Cu, Pb, Cd and Hg concentrations in the mantle of mussels /Unio sp./ collected from the open water of Lake Balaton

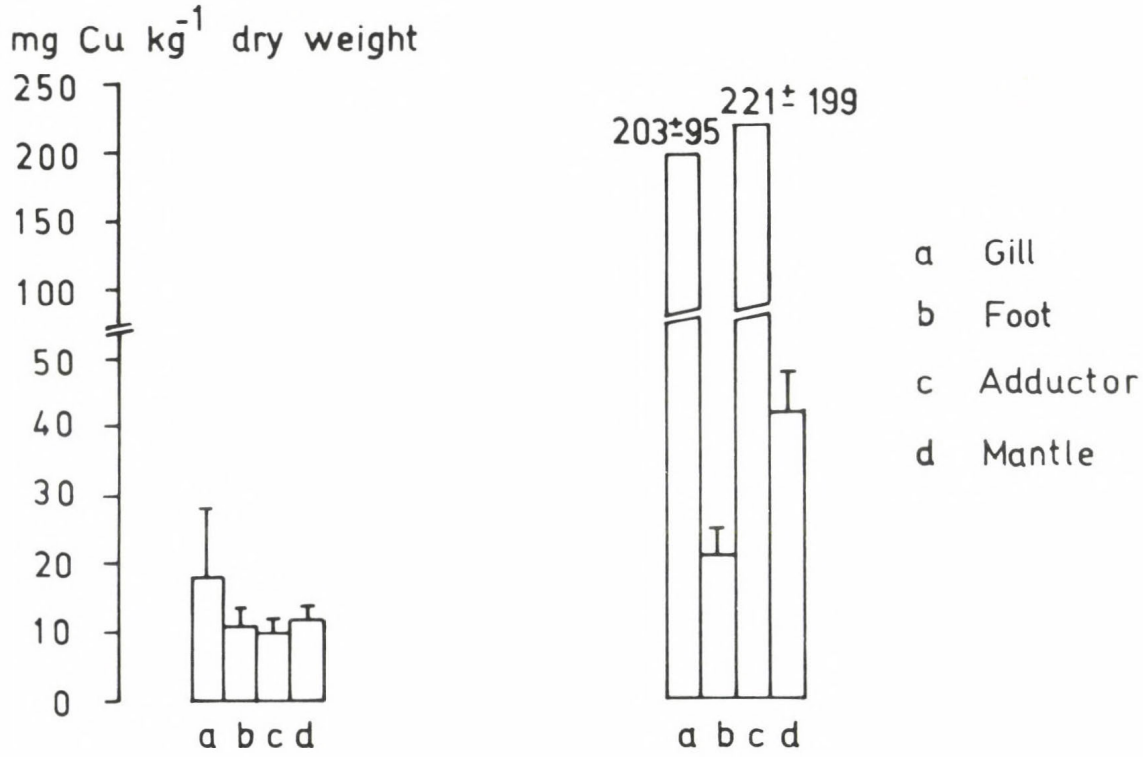

Fig. 11 - Copper concentration in various organs of mussels /Unio sp./ collected in the middle of the Lake /Station No 6/ and close to a sailing boat harbour /Station No 11/.

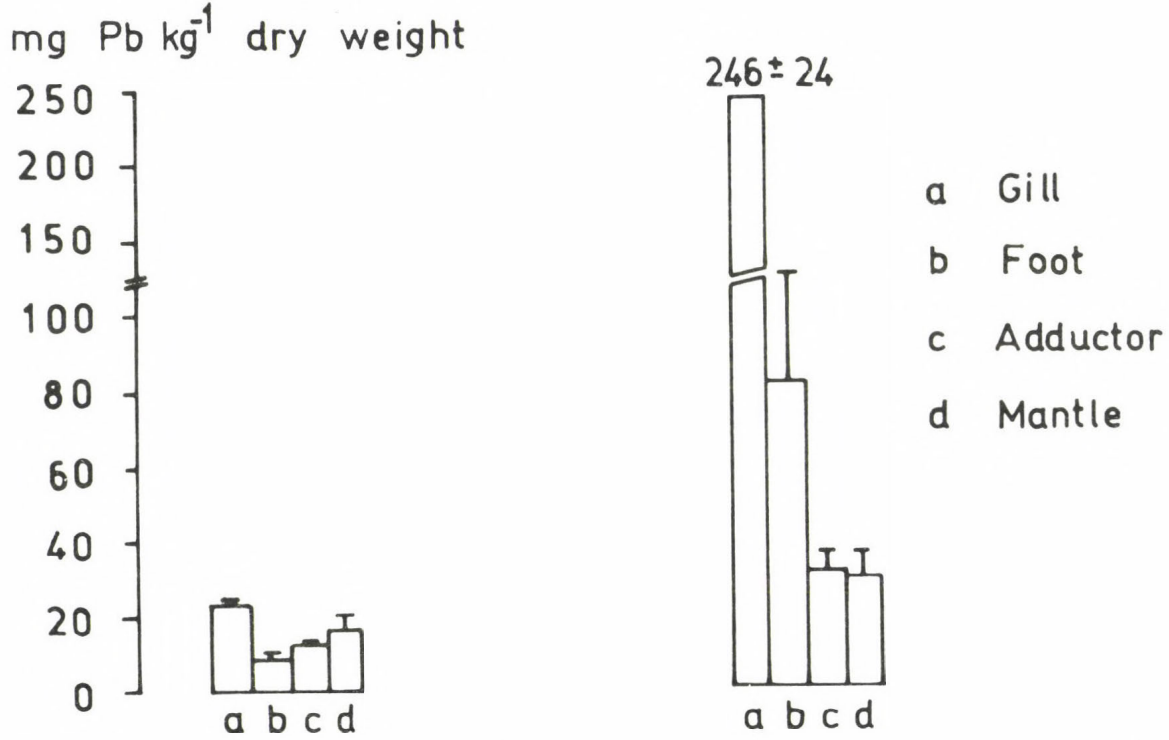

Fig. 12 - Lead concentration in various organs of mussels /Unio sp./ collected in the middle of the Lake /Station No 6/ and close to a sailing boat harbour /Station No 11/.

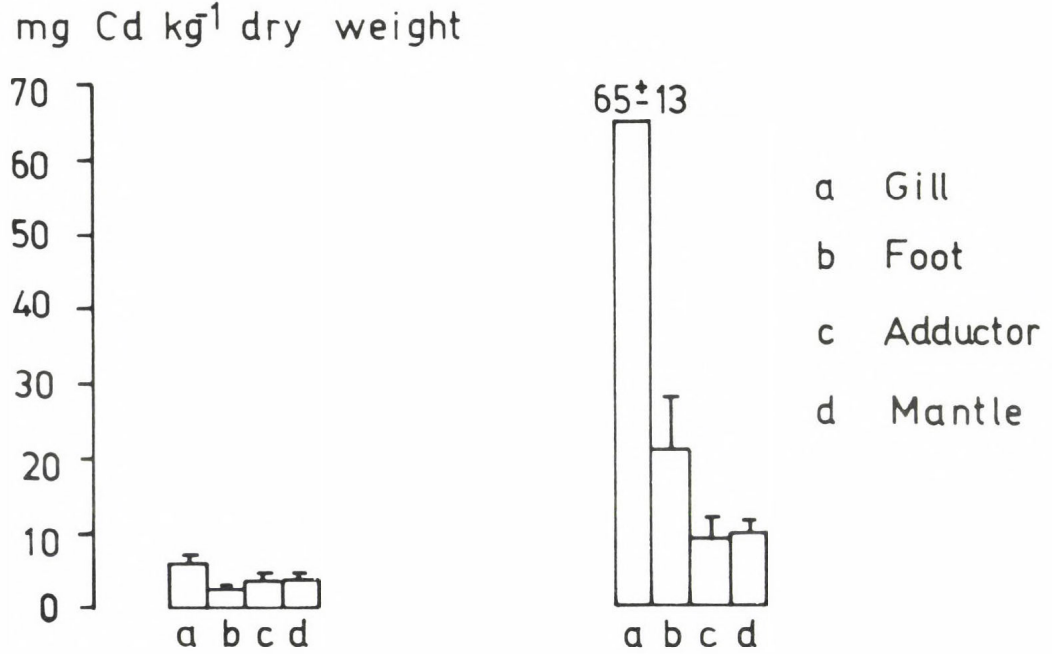

Fig. 13 - Cadmium concentration in various organs of mussels /Unio sp./ collected in the middle of the Lake /Station No 6/ and close to a sailing boats harbour /Station No 11/.

The high local concentration found in mussels close to the sailing boat harbour can be explained by pollution originating from paints which are used to protect boats and contain heavy metals. Some of these paints containing high concentration of copper are used for preventing the colonization of algae and other organisms on boats.

Looking for seasonal variations we found that concentrations of Hg and Pb were higher in crustacean plankton in summer, while Cd was lower in spring as compared to the other seasons. The origin of these differences can be interpreted as a result of abiotic influences, nevertheless, one should bear in mind that the species composition of the zooplankton may change with the season, further on, due to the temperature the lifetime of these animals may change /Cajander 1980, Horowitz and Presley 1977, P.-Zánkai 1978/. Nevertheless, crustacean plankton refers to a higher heavy metal pollution of the Lake in summer time than in spring and autumn.

Concerning heavy metal concentration in different organs of mussels the picture is somewhat different. Practically no consistent trends were detected parallel to the seasons. Similar findings were reported for mussels from different laboratories /Gault et al 1983, Goldberg et al 1978/. It seems probable that other variables than season play more important role in the determination of heavy metal concentration of mussels. Mussels are animals of long life span and age can be an important factor to be considered. We used about 6 to 8 years old animals in our measurements. Also the body weight of mussels can change with time, which may not be the same for the stored metals /Boyden 1974/. Nevertheless, we suppose that heavy metal variations in mussels are not the results of seasonal variations of animal metabolism, but it reflects temporal and local differences in the heavy metal concentration of the environment.

Finally, we conclude that both crustacean plankton and mussels are good indicators in monitoring heavy metal pollution of Lake Balaton, and such measurements should be repeated from time

to time to control whether the low contamination persists or changes with a further use and loading of the catchment area of Lake Balaton.

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DISCUSSION

FOWLER, S.W: Have you examined the possible relationship between water temperature and the seasonal maxima and

minima in heavy metal accumulation? The maxima you found in the summer could result from enhanced uptake of metal at higher temperatures which is well-known to occur in mussels.

V.-BALOGH, K: We did not examine the relationship between the water temperature and the heavy metal uptake or accumulation. The differences between the seasonal temperature maxima are not high enough to explain the summer uptake maximum. We took samples from April to September. The temperature maxima were 15°C as well as 24°C. We measured the highest Cd concentration in April in the foot and in the adductor muscles, which opposes the idea about the role of temperature in the described phenomenon.

WEIS, P: To continue along the line of thought brought up by Dr. Fowler, we have unpublished data on our fillifish /Fundulus heteroclitus/ that the beginning of the summer /25 May/ is associated with the highest concentration of Hg in both muscle and liver. At the end of summer /30 August/ the levels of Hg are lower, but the temperature is higher. Therefore, temperature is not necessarily a factor, but growth rate may be.

SHIBER, J.G.: Two points were considered:
1/ the size /length/ of the mussels analyzed
2/ whether the mussels were locally used as food.

V.-BALOGH, K: 1/ For analysis we used about 8 cm long mussels. 2/ These mussels are not edible.

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HEAVY METALS IN MARINE ORGANISMS

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Nowadays the pollution of water bodies has become an important problem because of urbanization and industrialization processes and intense agriculture activities. It concerns also the Baltic Sea. The basic pollution sources of the Baltic are the following: it is surrounded by economically highly developed countries discharging a great amount of technogenous substances into the sea, besides that atmospheric precipitation also adds a great deal (80-90%) to the marine environment. The so called anthropogenic factor has become the basic one not only in the pollution of the Baltic Sea but also in considerable changes of its biological ecosystem. The Baltic belongs under the inland seas being connected with the ocean through narrow sounds where the water exchange is slow and the pollutants accumulate mainly in the sea itself.

The compounds of heavy metals (mercury, lead, cadmium, zinc, copper, nickel, cobalt) appear to be the most dangerous pollutants having not only a direct toxic effect on the human and water organisms but also a dangerous mutagenous, embryotoxic and gonadotoxic after-effect, notwithstanding the fact that the level of metals is lower than that of the widely distributed oil products, chlorinated hydrocarbons and artificial radionuclides.

The present paper deals with the investigation results on the level of 13 metals (mercury, cadmium, cobalt, nickel, copper, lead, strontium, zinc, manganese, iron, magnesium, calcium) in the basic ecosystem elements (total plankton, benthic organisms, algae and fish) of the Gulf of Riga and separate parts of the open Baltics.

The level of the given metals, except mercury, was analysed by atomic absorption on spectrophotometer Perkin-Elmer-403 type. For our investigation objects various mixtures of acids were applied to extract the majority of elements from ash. Thus our conclusion is that the primary treatment should be done by a concentrated HNO_3 and the secondary by a mixture of HNO_3:HCl (1:3) reaching a maximum leaching of metals from the ash of aquatic organisms. Mercury was estimated by Coleman analyzer, type MAC-50. Water organism samples were prepared for wet ashing by

concentrated $HNO_3:H_2SO_4$ (1:1).

As it was mentioned already the investigations embraced the basic ecological groups of water organisms from above 25 areas. The sites of sample taking are presented in Fig.1.

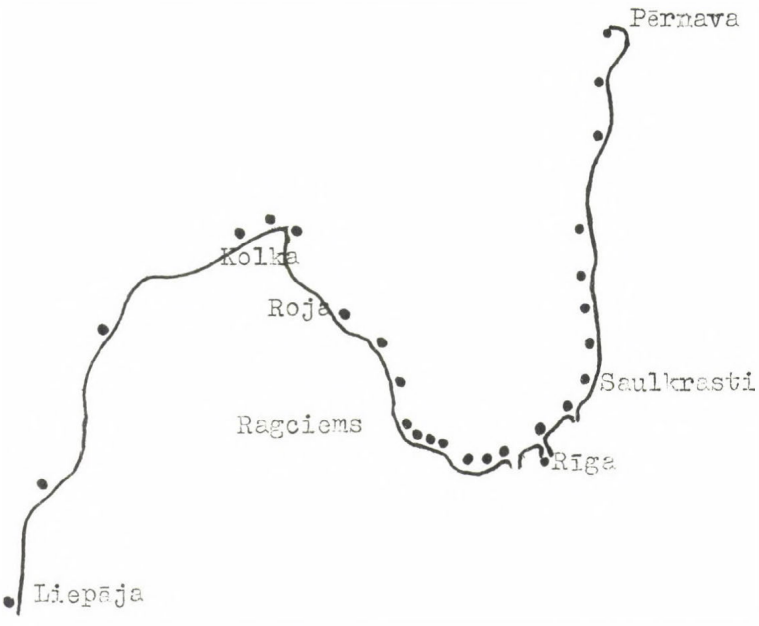

Fig.1. Dislocation of sampling sites of hydroorganisms in the Gulf of Riga and the open Baltic.

Plankton

The amount of mercury, cobalt, chromium, and cadmium stated in plankton is the lowest, then follows nickel, copper and lead their level being about 1 mg/kg of wet mass. Medium position is taken by strontium, zinc and manganese. The highest levels found in plankton belong to iron, magnesium and calcium, the last two being in similar amounts opposite to other water organisms where the level of calcium usually greatly exceeds that of magnesium. Qualitative and quantitative investigations of plankton revealed that difference in species composition greatly influenced the accumulation of separate metals in them. Cadmium and mercury accumulate more in zooplankton consisting mainly of cladocera forms - Podon sp., Bosmina obtusirostis. Maximum concentrations of chromium, lead, zinc, iron and magnesium are found in zooplankton consisting of copepods - Eurytemora and Acartia sp. The level of cobalt in various zooplankton did not show any differences being 0.2 mg/kg of wet mass in all the cases.

The average levels of the investigated metals in plankton of the Gulf of Riga are presented in Fig.2. According to our data the level of such metals as copper, lead, zinc, iron is lower, but that of chromium higher in the plankton of the

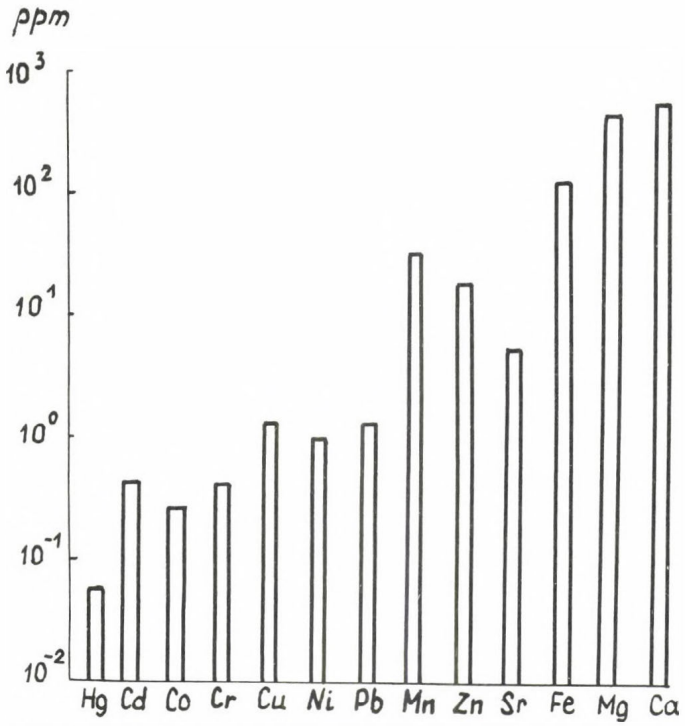

Fig.2. The level of metals in plankton (ppm wet mass).

Gulf of Riga that can be related to the differences in plankton composition and the time of investigation (2).

The following order of metals was obtained when comparing their levels in plankton:

Hg < Co < Cr < Cd < Ni < Cu < Pb < Sr < Zn < Mn < Fe < Mg < Ca

Mollusks

In bivalve mollusks the ability to accumulate metals from marine water is higher than in other water organisms.

Analysis of metal level accumulated by water organisms showed that the investigated three species of mollusks (Macoma baltica, Mytilus edulis, Mya arenaria) had an expressed ability to accumulate lead, cadmium, zinc, cobalt, chromium, nickel, iron, magnesium and calcium in larger amounts than other water organisms (Table 1).

The ability of bivalve mollusks to concentrate high levels of some metals from the marine water, their distribution, easy collection and low migration activity make some species (Mytilus edulis, Macoma baltica) suitable as bio-indicators - accumulators of metals in the monitoring of marine pollution.

Metal concentration in shells and in soft mollusk

Table 1

Level of metals in mollusks (ppm, wet mass)

	Hg	Cd	Co	Cr	Cu	Ni	Pb	Mn	Zn	Sr	Fe	Mg	Ca
Mya arenaria	0.006	6.1	22.8	11.3	4.5	34	33	25	71	1083	165	222	156334
Mytilus edulis	0.004	2.5	12.8	5.7	4.5	17	27	39	44	391	113	1018	92607
Macoma baltica	0.028	4.4	17.7	9.1	14.6	29	33	42	75	872	1378	407	160379

Table 2

Level of metals in benthic crustaceans (ppm, wet mass)

	Hg	Cd	Co	Cr	Cu	Ni	Pb	Mn	Zn	Sr	Fe	Mg	Ca
Mesidothea entomon	0.053	0.97	3.9	1.5	35.0	4.7	6.1	286	32	266	542	1667	26289
Neomysis vulgaris	0.012	0.12	0.4	0.3	4.8	0.9	1.1	10	16	38	108	675	4302

tissues is not equal according to the peculiarities of metal uptake by the organism, their circulation inside the organism and exit. In the shells of Macoma baltica and Mytilus edulis the amount of lead, cadmium, cobalt, chromium, nickel and mercury is higher than in the mollusks in general.

The amount of such metals as lead, nickel, cobalt, chromium, strontium and cadmium in Mya arenaria shells was considerably higher than that in the soft tissues that can be probably explained by the sedimentation character with carbonate material and fixation in the shells during their formation. In two types of mollusks Macoma and Mytilus edulis the level of metals was studied according to mollusk size. Almost in all the cases the level of metals in these mollusk species with different length of individuals was similar. Only in some cases the level of zinc was higher in mollusks of smaller size, and sometimes the same was observed with iron.

According to their concentration the following orders of metals were found in mollusks:

in Macoma baltica

Hg < Cd < Cr < Cu < Co < Ni < Pb < Mn < Zn < Mg < Sr < Fe < Ca

in Mytilus edulis

Hg < Cd < Cu < Cr < Co < Ni < Pb < Mn < Zn < Fe < Sr < Mg < Ca

in Mya arenaria

Hg < Cu < Cd < Cr < Co < Mn < Ni < Pb < Zn < Fe < Mg < Sr < Ca

Algae

In comparison with mollusks smaller amounts of iron, manganese, zinc, strontium, calcium and magnesium are found in algae, and the level of chromium, mercury and cadmium in them is very low. One of the factors determining such a difference in the accumulation of metals in algae is their specific variability to accumulate metals. All the metals studied were found in high amounts in Fucus vesiculosus and Furcellaria fastigiata and in low amounts in Cladophora glomerata and Enteromorpha intestinalis. Mercury was an exception showing maximum concentrations in the green algae in comparison with those in the brown algae. The level of metals in the green alga Enteromorpha intestinalis was studied simultaneously in various investigation areas (Fig.3). The results obtained prove that this algal species has a similar concentration of the majority of investigated metals in spite of the considerable distances among the sample taking areas.

Comparing the mean amounts of accumulated metals the following orders were obtained:

in the brown alga Fucus vesiculosus

Hg < Cr < Cd < Co < Cu < Pb < Ni < Zn < Sr < Mn < Fe < Mg < Ca

in the red alga Furcellaria fastigiata

Hg < Cd < Cr < Co < Ni < Pb < Cu < Zn < Mn < Sr < Fe < Mg < Ca

in the green alga Cladophora glomerata

Hg < Cd < Co < Cr < Cu < Pb < Ni < Zn < Sr < Mn < Fe < Mg < Ca

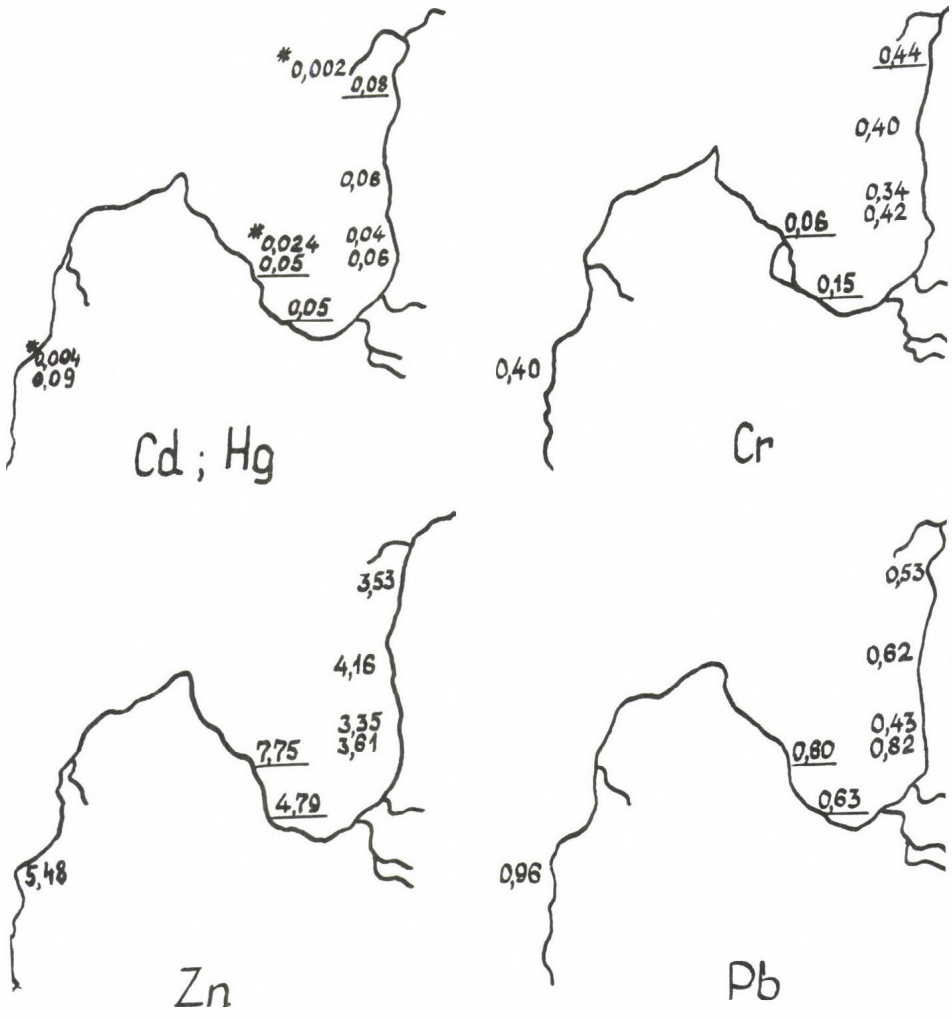

Fig. 3. Metal content (ppm wet mass) in the green alga Enteromorpha intestinalis from different areas of the Gulf of Riga and the open Baltic
*content of mercury

in the green alga Enteromorpha intestinalis

Hg < Cd < Co < Cr < Cu < Ni < Pb < Zn < Sr < Mn < Fe < Mg < Ca

Benthic crustaceans

Among the higher benthic crustaceans Neomysis vulgaris and Mesidothea entomon were studied according to the metal level accumulated in them. Maximum concentrations of mercury, lead, iron, zinc, copper, cobalt, chromium, nickel, manganese, magnesium and calcium were found in Mesidothea entomon (Table 2).

Such a selective accumulation of the majority of investigated metals is evidently related to the variability in chemical composition of biotope of these species, their way of life, metabolism and the difference in nutrition. In Mesidothea entomon, in comparison with all the other investigated water organisms, high amounts of copper were found. This metal is of a particular importance for the crustaceans. It appears to be a constituent part of hemocyanin molecule the latter acting as the basic pigment of respiration in crustaceans. This species is characterized also by high levels of manganese that can be related to the composition of sediments in the Gulf of Riga being rich in manganese concretions. The level of manganese in crustaceans is closely linked with the molting cycle as far as about 98% of manganese is contained by the exoskeleton as indicated by some authors (3).

In most cases there was no significant difference in metal levels in Mesidothea entomon and Neomysis vulgaris according to years. Comparing the levels of the accumulated metals in the investigated crustaceans the following orders were obtained:
in Mesidothea entomon

Hg < Cd < Cr < Co < Ni < Pb < Zn < Cu < Sr < Mn < Fe < Mg < Ca

in Neomysis vulgaris

Hg < Cd < Cr < Co < Ni < Pb < Cu < Mn < Zn < Sr < Fe < Mg < Ca

Fish and lamprey

13 fish species from the Gulf of Riga were analysed according to the metal level accumulated in them: the Baltic herring - Clupea harengus membras L., smelt - Osmerus eperlanus (L.), the Baltic sprat - Sprattus sprattus balticus (Schneider), vimba - Vimba vimba (L.), sig - Coregonus lavaretus (L.), eelpout - Zoarces viviparus (L.), fourhorn sculpin - Myoxocephalus quadricornis (L.), sand eel - Ammodytes lanceolatus (La Sauvage), flounder - Pleuronectes flesus L., ling - Rhombus maximus , ide - Leuciscus idus (L.), cod - Gadus morhua callarias (L.), pike perch - Lucioperca lucioperca (L.), bass - Perca fluviatilis, pike - Esox lucius, as well as lamprey - Lampetra fluviatilis (L.).

Comparing the mean amounts of the accumulated metals the following orders were obtained:
in the Baltic herring

Hg < Cd < Cr < Ni < Co < Cu < Pb < Sr < Mn < Zn < Fe < Mg < Ca;

in smelt
Cd < Hg < Cr < Ni < Co < Cu < Pb < Mn < Zn < Fe < Sr < Mg < Ca;

in the Baltic sprat
Hg < Cd < Co < Cr < Ni < Pb < Cu < Mn < Sr < Zn < Fe < Mg < Ca;

in eelpout
Hg < Cd < Cr < Ni < Co < Cu < Pb < Mn < Zn < Sr < Fe < Mg < Ca;

in fourhorn sculpin
Hg < Cd < Co < Pb < Cu < Ni < Mn < Zn < Sr < Fe < Mg < Ca;

in flounder
Cd < Cr < Ni < Co < Cu < Pb < Mn < Zn < Sr < Fe < Mg < Ca;

in ling
Cd < Cr < Cu < Co < Ni < Pb < Mn < Zn < Sr < Fe < Mg < Ca;

in sand eel
Cd < Cr < Pb < Cu < Ni < Co < Mn < Sr < Fe < Zn < Mg < Ca;

in ide
Cd < Cr < Cu < Co < Ni < Pb < Mn < Zn < Sr < Fe < Mg < Ca;

in cod
Hg < Cd < Cr < Co < Cu < Ni < Pb < Mn < Zn < Sr < Fe < Mg < Ca;

in bass
Cd < Cr < Cu < Ni < Co < Pb < Mn < Zn < Sr < Mg < Ca;

in pike
Cd < Cr < Cu < Co < Ni < Pb < Mn < Sr < Zn < Fe < Mg < Ca;

in pike perch
Hg < Cd < Ni < Cr < Cu < Co < Pb < Mn < Fe < Zn < Sr < Mg < Ca;

in lamprey
Hg < Cd < Cr < Pb < Mn < Co < Ni < Cu < Zn < Fe < Ca < Mg.

The Fig.4 presents the levels of the investigated metals in the main industrial fish species of the Gulf of Riga.

The amount of metals distributed in fish organs and tissues differs considerably. It is mainly localized in bones, gills, fins, liver, and spawn. The levels of calcium, magnesium, iron, and zinc are high in all the fish organs and tissues while the levels of cadmium and mercury are insignificant in them. Liver has a high concentration of copper, nickel, lead, zinc, iron, magnesium and calcium. The amount of copper in liver exceeds that in other organs and tissues by several times. According to our investigations of metal distribution, mercury is accumulated in muscles. Seasonal fluctuation in metal accumulation is best seen in separate organs (milt roe, spawn) than in the whole organism. The amount of metals in various organs and tissues appears to be a variable quantity closely depending on metabolism and a number of environmental factors.

Comparing the average amounts of the investigated metals in plankton-eating (B.herring, smelt, B.sprat), benthos-eating (eelpout, fourhorn sculpin, sand eel, flounder, ling, ide) and predator fish (cod, pike perch, pike, bass) it was found that average concentrations of cobalt, chromium,

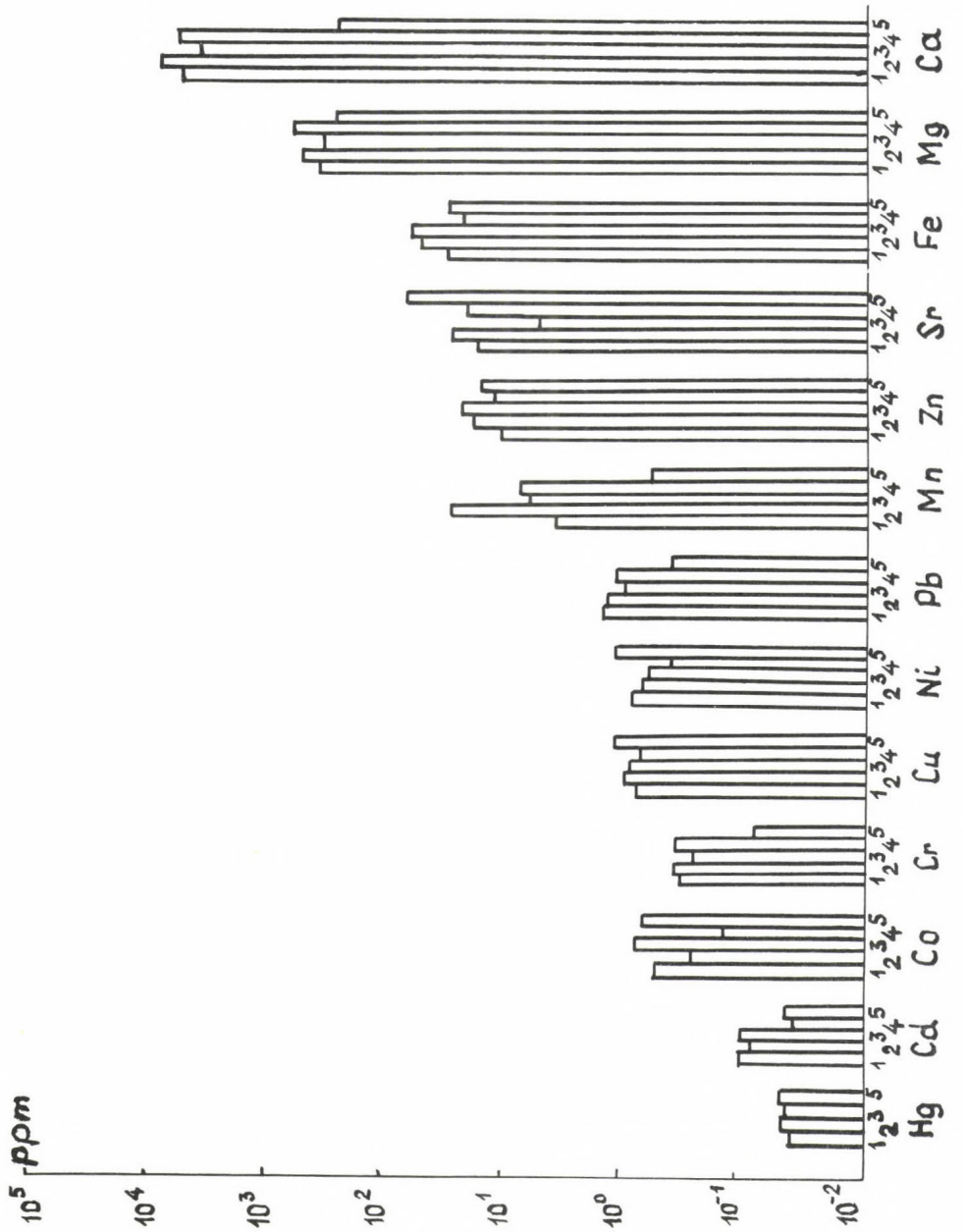

Fig.4. Metal content in industrially important fish species as well as lamprey
1 - cod; 2 - eelpout; 3 - Baltic herring;
4 - flounder; 5 - lamprey.

nickel and cadmium were similar in all the groups, the concentrations of copper, strontium and zinc were similar in benthos-eating and predator fish, and the concentrations of lead and magnesium were similar in plankton-eating and benthos-eating fish. Only the levels of manganese, iron and calcium differed in each fish group. In comparison with other fish species the predators have the highest levels of lead, magnesium and calcium that helps in the formation of their skeleton, but the level of manganese and iron in them is the lowest. Plankton-eaters are characterized by the highest concentrations of copper, zinc and iron, but benthos-eaters - by manganese. Mercury levels are lower in immature specimens of predators than in plankton-eaters and benthos-eaters.

Based on the whole material of data, the levels of all the investigated metals accumulated by water organisms can be divided into three groups:

1) very low (mercury, cadmium, chromium),
2) medium (nickel, lead),
3) high (iron, magnesium, calcium).

The results of our investigations on the accumulation of metals by water organisms annually demonstrate that the metal levels in them do not increase, but fluctuate within the same range (1).

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DISCUSSION

FOWLER, S: Have you related the measured metal concentration in organism to those in sea water? This would be useful to determine which metals are more bioavailable in a given species, a common method to evaluate potential indicator species.

SEISUMA, Z: In our present paper concentrations of metals are given only in the different marine organisms, but not in the water.Therefore coefficients of accumulation of these metals could not be determined in the organisms.

ECOLOGICAL MONITORING OF HEAVY METAL POLLUTION

Symposia Biologica Hungarica 29 (1985)

THE FREQUENCY OF BACTERIA RESISTANT TO HEAVY METALS IN PONDS OF SOUTHERN BOHEMIA

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Environment, including waters, is contaminated by highly toxic heavy metals /Förstner and Wittmann, 1983/. The contaminants are chiefly industrial and agricultural byproducts. The danger of toxic metals is incurred by the fact that the so-called biologically available part of the heavy metal in the environment is being transformed and accumulated by various organisms. Thus, heavy metals enter nutrition chains and potentially endanger living beings. In the processes of transformation and accumulation, micro-organisms participate primarily due to their considerable amount in the ecosystems and their great adaptability /Nelson and Colwell, 1975; Timoney et al. 1978; Baker et al. 1983/. Microbes can develop resistance to heavy metals, among others. The resistant bacteria display a series of mechanisms by which metals are transformed and detoxicated /Silver and Summers, 1978/. Resistance to metals /e.g., Hg, Cd, As, Sb, Zn, Ag, Cr, Sn/ has been ascertained in various bacteria living in soil and/or in water. The resistance may be a multiple one, i.e. a bacterium may be resistant to several metals and/or other toxic /e.g., antibiotic/ materials /Timoney et al. 1978/.

Olson and Thornton /1982/ has proposed that the tolerance of soil bacteria to metals may be used to determine the bioavailability of metals and their potential toxicity to other forms of life. Nelson and Colwell /1975/ concluded that the ratio of viable aerobic heterotrophic bacteria resistant to mercury to the total population of sea-water

bacteria positively correlated with the sediment mercury concentration and that the number of mercury-resistant bacteria expressed in colony-forming units can serve as a true index of in situ Hg^{2+} metabolism.

The present work was performed to compare the frequency of aerobic, mesophilic, heterotrophic bacteria resistant to mercury in various samples of freshwater ecosystems /waste water, water of ponds, sediment and water plant/ from the pond area in South Bohemia. The area is relatively clean and is contaminated predominantly with agricultural sources without industrial pollution.

MATERIAL AND METHODS

Surface and waste waters, sediments and various kinds of water and riparian plants were sampled in September 1983 in South Bohemia rich in ponds, in the vicinity of the town Třeboň in the basin of the River Lužnice. We have tested the waste water outflow from a farm of ducks /with a production of about half a million ducklings a year/. The waters of the system of ponds into which the outflow of the duckfarm entered without any transformation were tested, too. The outflow of the duck-farm passes through three ponds /main system/ called Sofl, Zdeňka and Čáp. The main system of the ponds is diluted by the relatively clear water of further ponds, i.e. Koubek, Rybl, Antonie and Zahrádka /diluting system/. For comparison we have tested the pond Černá, located in the forest territory in the vicinity of the system investigated, but without any connection with it.

Water samples were taken about 10 cm under the surface. The samples of water, sediments and water vegetation were taken into 500 ml sterile serologic bottles. In the laboratory the samples of surface water were immediately diluted decimally with sterile saline and inoculated /0.2 ml/ on medium in Petri-dishes.

The samples of plants were washed, cut into about 1 cm long pieces and washed again under running water for about

15 min. The washed samples were spread with 500 ml sterile saline. A hundred ml of sterile saline with 10 g of the sample in it was dried at 120°C for 10 h. Saline was added to sediment or water plants and the sample was homogenized. The homogenized sample was diluted decimally with saline.

The diluted and prepared samples of surface and waste waters, sediments and plants were poured on nutrient medium as described by Houba and Remacle /1980/. The medium was prepared without metal and with the following mercury chloride concentrations: 2, 5, 10, 15, 20, 50 and 100 μg $Hg \cdot ml^{-1}$.

The inoculated plates were incubated at 37°C for 24 h. After the results were read, colony numbers per 1 g dry matter were calculated. The Tables also indicate the percentages of the bacteria resistant to the given Hg concentration in the medium. Percentage was calculated by dividing the number of colonies /x100/ grown out in the presence of Hg by the number of colonies grown out in the absence of Hg.

RESULTS AND DISCUSSION

In Table 1 the numbers of viable bacteria resistant to Hg in waste water and its sediments are indicated. These waste waters, strongly contaminated microbiologically and chemically /Říha, 1983; Květ, 1984/ enter the pond Sofl without any treatment. In the pond, there are organisms capable of living in highly contaminated water.

Table 2 illustrates the resistance of bacteria sampled from water, sediments and plants on the border of pond Sofl. The total viable count is again very high at the dam of the pond as compared to the place where the waste water flows in from the duck-farm.

After leaving the ponds Sofl and Zdenka, the waste waters enter the pond Čáp, where they undergo self-cleaning and dilution and thus become less contaminated both chemically and microbiologically /Table 3 and 4/. The pond Černá has already regularly been planted with carp and there are plenty

Table 1 Counts of total and Hg-resistant bacteria in the waste water from duck-farm
Hg-resistant bacteria /CFU/ml of sample/

Date: 12.9.1983

	Water		Sediment	
$\mu g\ Hg\ ml^{-1}$ of medium		% of total		% of total
without Hg	1.55×10^6	100.00	5.22×10^7	100.00
1	1.00×10^6	64.52	1.50×10^7	28.74
2	2.75×10^5	17.74	2.28×10^6	4.37
5	2.00×10^4	1.29	4.18×10^5	0.80
10	1.40×10^4	0.90	3.63×10^5	0.70
15	1.50×10^4	0.96	2.13×10^5	0.41
20	5.00×10^3	0.32	1.00×10^5	0.19
100	0	0	0	0

Table 2 Counts of total and Hg-resistant bacteria in the pond Sofl
Hg-resistant bacteria /CFU/ml of sample/

Date: 12.9.1983

µg Hg ml^{-1} of medium	Water	% of total	Sediment	% of total	Plant-*Glyceria* sp.	% of total
without Hg	$7.50x10^6$	100.00	$2.56x10^7$	100.00	$9.64x10^5$	100.00
1	$5.00x10^6$	66.67	$3.00x10^6$	11.72	$7.14x10^5$	74.07
2	$5.00x10^5$	6.67	$2.37x10^6$	9.26	$7.14x10^4$	7.41
5	$3.35x10^4$	0.45	$9.59x10^4$	0.37	$1.08x10^4$	1.12
10	$6.00x10^3$	0.08	$7.78x10^4$	0.30	$9.64x10^3$	1.00
15	$3.50x10^3$	0.047	$2.50x10^4$	0.098	0	0
20	$1.00x10^3$	0.013	$1.67x10^4$	0.065	0	0
100	0	0	0	0	0	0

Table 3 Counts of total and Hg-resistant bacteria in the pond Čáp

Hg-resistant bacteria /CFU/ml of sample

Date: 12.9.1983

	Water		Sediment A		Sediment B	
$\mu g\ Hg\ ml^{-1}$ of medium		% of total		% of total		% of total
without Hg	9.5×10^3	100.00	1.37×10^5	100.00	9.64×10^5	100.00
1	5.0×10^3	52.63	1.29×10^5	94.16	3.67×10^5	38.07
2	8.6×10^2	9.05	1.29×10^5	94.16	3.57×10^5	37.03
5	5.0×10^2	5.26	3.15×10^4	22.99	6.07×10^4	6.30
10	5.0×10^1	0.53	1.45×10^3	1.06	5.24×10^4	5.44
15	2.0×10^1	0.21	1.37×10^3	1.00	9.64×10^3	1.00
20	0	0	1.05×10^3	0.77	5.00×10^3	0.52
100	0	0	1.61×10^2	0.14	0	0

Sediment A - near the pond Zdeńka Sediment B - near the pond Antonie

Table 4 Counts of total and Hg-resistant bacteria in the pond Čáp

Hg-resistant bacteria /CFU/ml of sample/

Dates	12.9.1983		26.9.1983			
	Duckweed /*Lemna* sp./		Duckweed /*Lemna* sp./		Plant-*Glyceria* sp.	
$\mu g\ Hg\ ml^{-1}$ of medium		% of total		% of total		% of total
without Hg	6.88×10^5	100.00	4.17×10^8	100.00	1.00×10^8	100.00
1	5.33×10^5	77.47	4.17×10^7	10.00	6.25×10^7	62.50
2	1.83×10^5	26.60	5.42×10^5	0.129	6.25×10^6	6.25
5	1.50×10^5	21.80	4.50×10^5	0.11	7.50×10^5	0.75
10	2.50×10^5	36.34	-	-	3.10×10^5	0.31
15	6.67×10^4	9.69	5.33×10^5	0.128	1.31×10^5	0.13
20	1.67×10^4	2.42	4.50×10^5	0.110	1.31×10^5	0.13
100	0	0	0	0	0	0

of water fowls feeding, among others, on duckweeds growing there in abundance. In Table 4 it can be seen that in the duckweeds the ratio of bacteria resistant to 2 μg Hg ml^{-1} concentration in the medium dropped within a fortnight from 26.6% to 0.13 %.

From the diluted system, the results for the pond Ryšl are shown /Table 5 and 6/.

In the relatively clear pond Černá, 0.64 to 17.1 % of the bacteria were resistant to 2 μg ml^{-1} Hg concentration. Bacteria did not grow in media of higher Hg concentration. In the sediment 4 % of the bacteria were resistant /Table 7/. In the reeds growing in the same pond 1.50-5.04 % proved to be resistant to the limit Hg concentration. There was no difference in bacteria resistance between roots and leaves of the reeds.

Summing up the obtained results, it can be shown that in the pond-waters 0.64 to 26 % of the bacteria were resistant to the Hg concentration 2 μg ml^{-1}. Bacteria resistant up to the concentration 20 μg ml^{-1} were found only in the pond Sofl, in its waste waters. In the other ponds of the main system the limit of resistance did not exceed 15 μg ml^{-1} Hg concentration in the nutrient medium.

In the ponds of the diluting system and in the pond Černá, resistant bacteria were found up to 2 to 10 μg Hg ml^{-1} at the utmost.

In the sediments 3.96-94.1 % bacteria resistant to 2 μg Hg ml^{-1} were ascertained. In the waste water of the pond Sofl, similarly to other waste waters, bacteria occurred resistant even to 20 μg Hg ml^{-1}. Bacteria resistant to 100 μg Hg ml^{-1} were found in the sediment at places where the contaminated waters of the pond Zdeňka enter the pond Cáp. In the diluting system and in the pond Černá the limit of resistance was 15 μg Hg ml^{-1}. The ratio of bacteria growing in the medium containing 2 μg Hg ml^{-1} was the lowest in the relatively clear pond Černá.

In the duckweeds 0.13-26.6 % bacteria resistant to 2 μg Hg ml^{-1} were found. The highest Hg concentration to which

Table 5 Counts of total and Hg-resistant bacteria in the pond Rýsl
Hg-resistant bacteria /CFU/ml of sample/

Dates:	12.9.1983		26.9.1983		12.9.1983	
	Water		Water		Sediment	
µg Hg ml^{-1} of medium		% of total		% of total		% of total
without Hg	$1.53x10^3$	100.00	$5.00x10^3$	100.00	$6.39x10^5$	100.00
1	$4.15x10^2$	27.12	$3.00x10^3$	60.00	$6.30x10^5$	98.59
2	$2.00x10^1$	1.31	$1.30x10^3$	26.00	$3.60x10^5$	56.33
5	0	0	0	0	$7.92x10^4$	12.39
10	0	0	0	0	$5.83x10^2$	0.09
15	0	0	0	0	$5.00x10^2$	0.08
20	0	0	0	0	0	0
100	0	0	0	0	0	0

Table 6 Counts of total and Hg-resistant bacteria in the pond Ryšl

Hg-resistant bacteria /CFU/ml of sample/

Dates:	26.9.1983		12.9.1983		12.9.1983	
	Duckweed /*Lemna* sp./		*Spirodella* sp.		*Elodea* sp.	
$\mu g\ Hg\ ml^{-1}$ of medium		% of total		% of total		% of total
without Hg	3.13×10^{8}	100.00	4.00×10^{7}	100.00	8.08×10^{7}	100.00
1	6.25×10^{6}	2.00	6.25×10^{6}	15.63	4.17×10^{7}	51.61
2	7.25×10^{5}	0.23	6.25×10^{6}	15.63	4.17×10^{7}	51.61
5	5.00×10^{4}	0.016	1.00×10^{6}	2.50	4.17×10^{7}	51.61
10	5.00×10^{4}	0.016	6.25×10^{5}	1.56	4.17×10^{7}	51.61
15	3.25×10^{4}	0.010	6.25×10^{5}	1.56	4.17×10^{7}	51.61
20	7.50×10^{3}	0.002	6.25×10^{4}	0.16	6.67×10^{6}	8.25
100	0	0	0	0	0	0

Table 7 Counts of total and Hg-resistant bacteria in the comparative pond Černá

Hg-resistant bacteria /CFU/ml of sample/

Dates:	12.9.1983 Water		26.9.1983 Water		12.9.1983 Sediment	
µg Hg ml^{-1} of medium		% of total		% of total		% of total
without Hg	$1.75x10^3$	100.00	$5.50x10^3$	100.00	$3.84x10^6$	100.00
1	$5.00x10^2$	28.57	$2.55x10^2$	4.64	$2.59x10^6$	67.45
2	$3.00x10^2$	17.14	$3.50x10^1$	0.64	$1.52x10^5$	3.96
5	$5.00x10^1$	2.86	0	0	$1.18x10^5$	3.24
10	$1.50x10^1$	0.86	0	0	$1.06x10^5$	2.76
15	0	0	0	0	$2.34x10^4$	0.61
20	0	0	0	0	0	0
100	0	0	0	0	0	0

bacteria were resistant was 20 μg ml^{-1} in almost all of the plants examined. The highest frequency of resistance /51.6 %/ was found in the Elodea species. In the other species 1.50 to 7.41 % of the bacteria were resistant to 2 μg Hg ml^{-1}.

A preliminary chemical analysis shows that the concentration of mercury in water phase is very low - in the range of 0.02 - 0.16 μg Hg l^{-1} of water. Hg concentration is between 0.042 and 0.144 μg Hg g^{-1} of dry sediments and water plant, 0.002 - 0.760 μg g^{-1} of dry matter of plants /D. Zachardová - personal communication/.

It is remarkable that the Hg concentrations tolerated by certain bacteria living in the area under study are 10^4-10^5 higher than the concentrations in the waters they inhabit. Some results for another locality agree with this result /Říha et al. 1983/. Presumably, the resistant bacteria derive from the sediments, for the limit concentrations to which bacteria are resistant are comparable to the Hg concentrations present in the sediments. It is probable that the resistant strains are selected on the effect of the high Hg concentration in the sediments. The resistant strains can metabolize Hg in the sediments and release it into the water-phase in various forms /Nelson and Colwell, 1975; Förstner and Wittman, 1983/.

The role of epiphytic bacteria growing on water plants is not known sufficiently. They participate in nitrogen fixation and other, little-known processes /Zuberer, 1984/. The water plants can accumulate various toxic metals, but the role of epiphytic bacteria in the process of accumulation and transformations is unknown. Our results show that a part of the population of epiphytic bacteria is resistant to 1-20 μg Hg ml^{-1}. On the duckweeds /Lemna/ 0.13 - 26.6 % of the bacteria were found resistant to 2 μg Hg ml^{-1} in the medium. The highest frequency of resistant bacteria was found in the species Elodea - 51.6 %.

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DISCUSSION

OZIMEK, T: 1/ Is there a trophic relation between epiphytic bacteria and the plants?

2/ Do you think that epiphytic bacteria can protect plants against mercury?

ŘIHA, V: 1/ I suppose that there exist trophic relations between epiphytic bacteria and water plants but according to the literature we have only proof that epiphytic bacteria associated with water plant can fix nitrogen. Other relations are unknown.

2/ This is a very interesting question stimulating further research. It is very hard to say because we have no proof, but I suppose that epiphytic bacteria can protect water plants against several toxic metals in specific conditions. This hypothesis is based on the knowledge of some bacterial mechanisms by which they detoxicate their own environment and on their capability to bioaccumulation.

SALÁNKI, J: 1/ What were the criteria for resistance? Do they just survive, do they reproduce, or do they need the Hg for their life?

2/ Since these bacteria are capable of liberating Hg from the sediment, the ecological function of mercury-resistant bacteria can be considered useful or undesirable from the point of view of environmental protection?

ŘIHA, V: 1/ For our investigation we have a criterion that these strains of tested bacteria can grow on a medium containing 2 μg of mercury /as $HgCl_2$/ ml^{-1} or more.

2/ It is difficult to state whether the role of bacteria is useful or undesirable. These bacteria can detoxicate their own environment, primarily by the process of biomethylation. By this process they clean the environment from the Hg^{2+}, but the methyl-derivate of mercury is usually more dangerous for other forms of life living in waters.

BOROSS, L: The authors have shown that the bacteria which were resistant to Hg^{2+} contained only a small amount of Hg^{2+}. Furthermore, about the same part of the population was resistant towards Cd^{2+}. Do these strains have a special barrier system against the heavy metal ions, or do they contain a special active membrane transport system to exclude some

toxic heavy metal ions? Do the authors study the rate of the uptake of mercuric ion by these bacteria in a model system?

ŘÍHA, V: We have not studied the rate of uptake of mercuric ions as yet on a model system. We studied only the frequency of bacteria resistant to mercury and cadmium.

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TOTAL MERCURY CONTENT IN THE COMPONENTS OF RUNNING WATER, RESERVOIR AND POND ECOSYSTEMS IN CZECHOSLOVAKIA

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Fishes as the final link of the food chain in aquatic environment are the main bioindicators of water pollution. Therefore it was just the ichthyotoxicology to which the attention was focused in the framework of the first phase of aquatic toxicology development in Czechoslovakia. The present phase of development is characterized by the complex solution of problems, not only from the point of view of fish protection, but also from the point of view of aquatic environment protection. This tendency of development is reflected also in the investigations of content of toxic metals, particularly mercury, in aquatic environment. Total mercury content analysis in fishes has been realized in the Research Institute of Fishery and Hydrobiology at Vodňany since 1973. Recently the content of mercury is being evaluated also in the other components of aquatic ecosystem. This paper presents results of total mercury content studies in fishes and in the other aquatic environment components of the Berounka river and selected fish-ponds.

The content of total mercury in the components of aquatic environment was determined by flameless atomic absorption method after the previous mineralization of samples in closed system by means of nitric acid and sulphuric acid mixture /STUDNICKA et al. - 1974/.

Investigation of total mercury content in individual components of aquatic ecosystem was realized at 12 localities of the Berounka river catchment area. A total of 1187 individuals of 23 fish species were analysed. Differences in total mercury

content in fish musculature from different localities were determined. The highest content was determined in fishes from localities below Pilsen, the lowest one from localities above Pilsen and Prague. The average value of total mercury content in most fish species ranged between 0.10 - 0.30 mg.kg^{-1} of musculature. Concentration of 0.5 mg.kg^{-1} Hg was exceeded only in pike, perch, pikeperch, asp and - in some localities - in barbel. Average values from 0.30 to 0.50 mg Hg per 1 kg of musculature were determined primarily in barbel. Average values 0.10 - 0.30 mg.kg^{-1} Hg referred to most fish species and concentrations below 0.10 mg.kg^{-1} Hg were sporadically detected in carp, tench, brown trout and rainbow trout.

Referring to the Czechoslovak directions on extraneous substances in food products, valid since 1979, concentration of 0.1 mg.kg^{-1} Hg is fixed as a maximum admissible level of mercury content in musculature of freshwater fishes. Based on results of analyses, nearly all fish species from localities of the Berounka river exceeded this admissible level.

As to species diversity in total mercury content in musculature of fishes from the Berounka river localities /table 1/, the highest mercury content was found in predatory fish species, followed by benthos feeders /barbel, dace/. This species diversity is connected with the different mercury content in the consumed food /MEYER - 1972, OTTE et al. - 1973/. In the Berounka river basin with a shortage of predatory fish species, the barbel can serve as a suitable bioindicator of environment pollution by mercury. The ability of barbel to accumulate mercury was found also in other localities /PEŇÁZ et al. - 1979, SVOBODOVÁ, HEJTMÁNEK - 1976, KNÖPPLER, DORN - 1976/. Correlation between total mercury content in musculature and weight and/or age of fishes was found in some fish species /perch, dace, gudgeon, roach/.

Mercury content determined in surface water is not quite decisive indicator of the extent of water environment pollution. Mercury transits from water into the bottom sediments of streams and reservoirs where it mostly accumulates in form of sulphide. Total mercury content in water samples from localities of the Berounka river basin ranged between 0.03 -

- 0.16 $\mu g.l^{-1}$. The determined values of mercury refer to the natural concentration in waters /WINKLER - 1975, NABRZYSKI - 1975/ and they are about one order lower compared with the admissible mercury content in drinking water /Czechoslovak Standard No. 83 0611/. Total mercury content in the samples of bottom sediments from the localities of the Berounka river basin ranged between 0.18 - 3.66 $mg.kg^{-1}$ Hg in dry matter of sediment. Total mercury content in sediments depends mainly on character of sediment. Samples with prevalence of mud and organic substances mostly revealed higher mercury content compared with samples of sand character. Therefore the role of sediment in bioindication of mercury pollution is a disputed matter in the studied localities of the Berounka river.

As to the other components of the Berounka river water ecosystem, water plants, algae, moss mats, mayflies, sedge flies and leeches were analysed for total mercury content. In addition, content of digestive tract of fishes was also analysed. Results of these analyses are presented in table 1.

Total mercury content in the basic components of the Berounka river ecosystem:

Analysed sample	unit	total mercury content
Predatory fishes	$mg.kg^{-1}$ /musculature/	> 0.50
Barbel	"	0.30 - 0.50
Other fishes	"	0.10 - 0.30
Water	$\mu g.l^{-1}$	0.03 - 0.16
Bottom sediments	$mg.kg^{-1}$ /dry matter/	0.18 - 3.66
Macrovegetation /*Ranunculus fluitans*/	$mg.kg^{-1}$	0.005 - 0.062
Algae	"	0.005 - 0.024
Moss mats /Fontinalis sp./	"	0.016 - 0.062
Mayflies /Ephemeroptera/	"	0.02 - 0.06
Sedge flies /g. Hydropsyche/	"	0.02 - 0.06
Leeches /*Helobdella stagnalis*/	"	0.025 - 0.087

Total mercury content in these components of water ecosystem was 10 times lower compared with the values for fish musculature. This coefficient of accumulation is comparable with food coefficient of fishes consuming the live invertebrates. The highest mercury content was found in leeches /Helobdella/ which can be explained by the predatory way of life and by the connection with the studied environment during the whole life period. Referring to the abundant occurrence of leeches in different types of aquatic environment, these species /together with mayflies and sedge flies/ can be considered as valuable indicator of mercury pollution. Total mercury content in digestive tract of fishes from the localities of the Berounka river basin referred to the total mercury content in natural food of fishes /0.02 - 0.08 $mg.kg^{-1}$/.

Content of total mercury in organs and tissues of carp, water, bottom sediments, zooplankton, zoobenthos and macrovegetation in fish-ponds with different basin character was also studied in the course of the growing season. These studies included the following fish-ponds: the Spolský pond /with prevailing agricultural water catchment area/, the Ruda pond in the Třeboň region /with prevailing forest water catchment area/, the Labská pond /with sewage waters and power-plant waste/, and the Újezdský pond in the Pardubice region /with power-plant and industrial wastes/. Musculature, liver and kidney were analysed in carp. Zooplankton samples were formed mostly by the mixture of Cladocera and Cyclops, Chironomidae dominated in zoobenthos. Sample of macrovegetation was represented by Glyceria aquatica in the Spolský and Ruda ponds, by Phragmites communis and Ceratophyllum demersum in the Labská pond, and by Potamogeton gramineus in the Újezdský pond.

The results of analyses are presented in tables 2 and 3. Based on total mercury content, the rank of individual carp organs and tissues was as follows: musculature > kidney > liver. Values found in musculature were significantly higher /$P<0.01$/ as compared with the values in kidney and liver. The maximum admissible concentration 0.1 $mg.kg^{-1}$ of mercury in fish musculature was never exceeded. The values of total mercury con-

Table 2: Total mercury content /mg.kg^{-1}/ in fresh tissue of carp C_{1-2} and C_{2-3} from selected fish-ponds in 1980

Pond		Date of sampling							
		May		June		August		October	
		n	$\bar{x}\pm s_{\bar{x}}$	n	$\bar{x}\pm s_{\bar{x}}$	n	$\bar{x}\pm s_{\bar{x}}$	n	$\bar{x}\pm s_{\bar{x}}$
Spolský	weight of fish/g/	20	415±36.4	20	653±37.8	16	770±66.9	15	1070±70.6
	musculature	20	0.035± 0.0032	20	0.017± 0.0011	14	0.017± 0.0015	15	0.023± 0.0010
	liver	20	0.017± 0.0022	18	0.009± 0.0004	13	0.018± 0.0018	15	0.012± 0.0007
	kidney	18	0.019± 0.0008	18	0.013± 0.0005	16	0.022± 0.0016	15	0.014± 0.0007
Ruda	weight of fish/g/	20	435± 43.9		603±43.7	15	776±71.7	16	1127±68.9
	musculature	19	0.034± 0.0017		0.025± 0.0015	15	0.027± 0.0013	16	0.027± 0.0013
	liver	20	0.016± 0.0011		0.018± 0.0008	15	0.016± 0.0007	16	0.010± 0.0006
	kidney	20	0.017± 0.0008		0.017± 0.0009	15	0.017± 0.0007	16	0.012± 0.0006
Labská	weight of fish/g/	27	18.7 ± 0.916			31	85.0 ± 4.54	31	153±10.5
	musculature	27	0.049± 0.0035			31	0.021± 0.0007	31	0.011± 0.0012
	liver	13	0.052			26	0.017± 0.0012	30	0.006± 0.0003
	kidney					22	0.021± 0.0016	31	0.006± 0.0001
Újezdský	weight of fish/g/							29	177±11.5
	musculature							27	0.026± 0.0016
	liver							29	0.008± 0.0003
	kidney							29	0.009± 0.0003

Table 3: Total mercury content in the other components of fish-pond water environment in 1980

Pond	Component		Date of sampling			
			May	June	August	October
Spolský	water	$\mu g.l^{-1}$	0.060	0.040	0.120	0.040
	sediment	$mg.kg^{-1}$/dry matter/	0.050	0.063	0.067	0.099
	zooplankton	$mg.kg^{-1}$/fresh tissue/	0.022	0.005	0.008	0.007
	zoobenthos	$mg.kg^{-1}$/fresh tissue/	0.013	0.009	0.008	0.017
	macrovegetation	$mg.kg^{-1}$/fresh tissue/		0.007		
Ruda	water	$\mu g.l^{-1}$	0.060	0.060	0.050	0.025
	sediment	$mg.kg^{-1}$/dry matter/	0.024	0.067	0.145	0.130
	zooplankton	$mg.kg^{-1}$/fresh tissue/	0.026	0.007	0.008	0.007
	zoobenthos	$mg.kg^{-1}$/fresh tissue/		0.013	0.013	0.019
	macrovegetation	$mg.kg^{-1}$/fresh tissue/		0.011		
Labská	water	$\mu g.l^{-1}$	0.100		0.080	0.020
	sediment	$mg.kg^{-1}$/dry matter/	0.160		0.132	0.098
	zooplankton	$mg.kg^{-1}$/fresh tissue/	0.007		0.006	0.024
	zoobenthos	$mg.kg^{-1}$/fresh tissue/	0.012			
	macrovegetation	$mg.kg^{-1}$/fresh tissue/			0.007	
Újezdský	water	$\mu g.l^{-1}$			0.070	0.110
	sediment	$mg.kg^{-1}$/dry matter/			0.072	0.144
	zooplankton	$mg.kg^{-1}$/fresh tissue/			0.007	0.049
	zoobenthos	$mg.kg^{-1}$/fresh tissue/				0.039
	macrovegetation	$mg.kg^{-1}$/fresh tissue/			0.015	

tent in water were low in all ponds and corresponded to natural mercury concentration in waters /WINKLER - 1975/. The average content of total mercury in samples of bottom sediments ranged between 0.024 - 0.160 $mg.kg^{-1}$ of dry matter. These values are about one order lower compared with the concentrations in stream sediments in Czechoslovakia /SVOBODOVÁ et al. - project report, 1979/. Based on total mercury content, the rank of the other fish-pond ecosystem conponents was as follows: zoobenthos› zooplankton› macrovegetation. Compared with values for fish musculature, total mercury content in food components is significantly lower. Content of total mercury in applied feed ranged between 0.01 - 0.05 $mg.kg^{-1}$ in all studied ponds.

The results of study documented the low mercury pollution of fish-ponds with different catchment area character. In these ponds, the mercury content in water was low and the differences among individual ponds were insignificant. Hypothesis on increased contamination of pond environment with prevailing agricultural catchment area was not proved. Increased concentrations of mercury were not detected even in individual components of water biocenosis in the Labská and Újezdský ponds loaded with power-plant and industrial wastes.

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DISCUSSION

WACHS, B: Fish as an indicator of mercury pollution in rivers is a problem. In general the Hg concentration in muscle tissues increases with increasing water Hg values, but there is a wide range of the accumulation size. From a bio-indicator we expect that it will be sensitive enough to indicate rather low differences of Hg concentration in water. This is not the case with fishes, but it is possible with averaged data of mosses /Fontinalis/, filamentous green algae or luches /Erpoldella/, Asellus, or gammaridae for example.

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BIOINDICATORS FOR THE HEAVY METAL LOAD OF RIVER ECOSYSTEMS

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INTRODUCTION

In determining the metal load of rivers for the purpose of surveilling and improving water quality, one basic question is which of the following sample types should be analyzed:

- water
- sediments
- fish
- zoobenthos
- aquatic plants.

A prerequisite for any kind of meaningful assessment is the knowledge of the background level for each type of sample material analyzed.

The determination of the average metal concentrations in water which are caused by emissions is difficult. Because of frequent changes in emission levels and fluctuations in river flow, continuous sampling of the water is almost indispensable.

Sediments, and especially the finest grain size fractions, show the highest accumulation factors of all potential sample materials in aquatic ecosystems. The analysis of sediments yields useful information on the metal burden of natural waters. Especially in the presence of a high load of organic material /1/, the level of metal contamination can be assessed by analyzing suspended matter and sediments because solid material acts as a sink for heavy metals. There are problems with this type of sample material, however, since the relevance of analytical re-

sults depends on a number of hydrogeochemical factors as well as on methodological details (c.f. /2/). For example, results are easily affected by changes in water chemistry. The isolation of defined and comparable particle size fractions (e.g., clay size fraction) is time consuming and often hampered by technical difficulties (e.g. /3/). Despite these difficulties, grain size fractionated samples have to be used when the time history of fluctuating metal loads needs to be established. Two essential prerequisites for such investigations are the presence of unperturbed sedimentary deposits and a sampling technique which does not disrupt the depth profile of the sediment cores.

BIOACCUMULATION

The absolute concentrations of the most important metals in fish muscle tissue decrease in the following order:

Zn >> Cu > Pb ≥ Hg >> Cd.

This series is based on analyses carried out in rivers with an average metal load over a period of many years. Among the different organs analyzed, the kidney showed the highest Cd and Zn concentrations, whereas Cu was preferentially enriched in the liver and Hg in muscle tissue. A comparison of metal concentrations in several organs produced the following general sequence for freshwater fish (organs: B = brain, G = gills, K = kidney, L = liver, M = muscle, S = spleen):

Hg:	M	>	S	>	K	>	L	>	B	> G
Cu:	L	>>	K	>	S	>	B	>	G	> M
Cd:	K	>>	L	>	S	>	B, G, M			
Zn:	K	>	L	>	S	>	G	>	B	> M
Fe:	K	>	L	>>	M					

With fish from rivers with a considerable mercury burden (e.g., typical values for one of our study objects are 0.5 - 2.5 µg/l dissolved Hg and 40 - 150 mg/kg Hg in dried mud) higher mercury concentrations are found in all organs, but at those elevated levels Hg is predominantly stored by the liver and kidney. The aforementioned accumulation series for the larger organs is essentially reversed; according to our experience it then

reads:

$$L > K \geq S > B \geq M > G$$

This brief outline of the accumulation behavior of metals in fish shows very distinctly that the kidney, liver and spleen react much more strongly to metal pollution than muscle tissue. Due to functional differences between the organs, the relative accumulation values can vary depending on the types of metals present in the water. For some elements, the liver is a better indicator of an elevated metal load, while for others it is the kidney [4]. Muscle tissue of fish caught in rivers with average to moderate metal loads, i.e., outside of massively polluted areas, has no indicator function at all [5 - 7]. Elevated metal concentrations found in gill books reflect situations of acute pollution since the metals are fixed by adsorption processes which occur very rapidly.

Based on the described results of metal concentration measurements in fish and on present knowledge of the biomagnification behavior of metals, it can be concluded that fish, which are end members of the aquatic food chain, cannot serve as sensitive indicators of the metal load of river ecosystems. With respect to heavy metals as pollutants it has not been possible to prove the biomagnification hypothesis predicting a successive accumulation of metals along the food chain [4, 6, 12]. Although fish are final consumers in this chain, their muscle tissue shows lower metal concentrations than zoobenthic organisms [5 - 11]. In natural waters with typically much lower pollutant concentrations than those used in laboratory experiments, the uptake routes water ——> benthos and water ——> fish are generally dominant and prevent the food chain effect from gaining any analytical significance [5 - 7].

In aquatic systems, metal ions or compounds in solution are available for biota by adsorption on the surface of organisms and by translocation into the cells. With animals, an important pathway is the uptake through the skin and gills. Metals in particulate form or insoluble compounds are ingested by animals together with their food. Accumulation of metals is generally more pronounced with submerged than with emerging or

floating plants. This is probably due to the fact that aquatic plants fix metals predominantly by adsorption on their surface. In contrast, some members of the aquatic fauna can actively take up metals through their skin. Mayfly larvae, for example, are covered with special ion-transporting chloride cells which accumulate iron and zinc [13]. Individual heavy metals enter organisms at different kinetic rates and have different residence times once they are incorporated. This has a pronounced effect on the results of chemical analyses of the organisms.

For the purpose of this study, bioaccumulation shall be understood to include adsorbed as well as incorporated metals; i.e., the analytical results were obtained on organisms not washed with dist. water. Our extensive measurements on species from numerous Bavarian rivers have shown that for the majority of fluvial organisms the absolute metal concentrations follow this series:

$$Cu > Pb >> Cd > Hg.$$

A comparison of the element-specific ability of aquatic plants and invertebrates to accumulate different heavy metals in terms of concentration factors, i.e. the relative concentration increases with respect to the surrounding water, yielded the following general accumulation series:

a) for submerged flora: $Cu >> Pb > Hg \geq Cd$

b) for macrozoobenthos: $Cu >> Hg \geq Cd > Pb.$

The metal concentrations in biological material collected at the same sampling site can fluctuate widely. This is most likely due to changes in the adsorption and desorption processes in the sediments, which in turn are affected by shifting grain size distributions and changes in the organic content of fluvial deposits. Another major reason for the rather wide range of concentration values found for a single plant species at a given sampling site is the fact that the collected plants may be at different stages of their development. The metal content of different organs changes during the course of the vegetation period [14, 15]. In addition, there is usually a distinct metal distribution among the permanent organs. This distinction is quite obvious in higher aquatic plants because of

their differentiation into leaf, stalk and root parts [7, 16 - 18]. Whereas the metal content of sediments tends to equilibrate with that of the water and the accumulation by submerged aquatic plants is mostly controlled by adsorption processes, the levels and distribution of metal concentrations in organisms of the aquatic fauna are subject to regulatory and detoxification mechanisms. The biological regulatory systems are more effective in maintaining the organic health of organisms the more highly developed the aquatic animals are.

BIOINDICATION

Analyses of benthic algae, macrophytes, invertebrates and fish have provided us over the years with comprehensive data material regarding the average extent of bioaccumulation of environmentally important metals which reflects the concentration levels of those metals in water bodies. Since a series of aquatic organisms concentrate metals in larger amounts, the corresponding genera are suitable bioindicators in locating continuous emissions and in delineating contaminated stretches of rivers as well as in the reliable surveillance of those stretches in the future [4, 7, 12]. Based on our extensive measurements in variably contaminated rivers, members of the aquatic macroflora and fauna were arranged in suitability sequences according to the relative increase in bioaccumulation [7]. Figures 1 - 3 present the sequences in the form of an accumulation spiral (cf. [4]) or accumulation series. Within the bioindication sectors shown in the figures, the indicator value of the organisms increases with increasing distance from the center. Figure 1 shows the general ranking of indicators separately for the aquatic flora and fauna. The indicator list of frequent fish food species in the lower half of Fig. 1 is lead by *Asellus*, followed by gammaridae, and finally by leeches of the genus *Erpobdella*. In listing the bioindicators which are useful for practical and routine investigations in Figs. 2 and 3, less frequent or rare species were omitted. Only those organisms were included which occur widely and can be found in sufficient numbers for meaningful sampling.

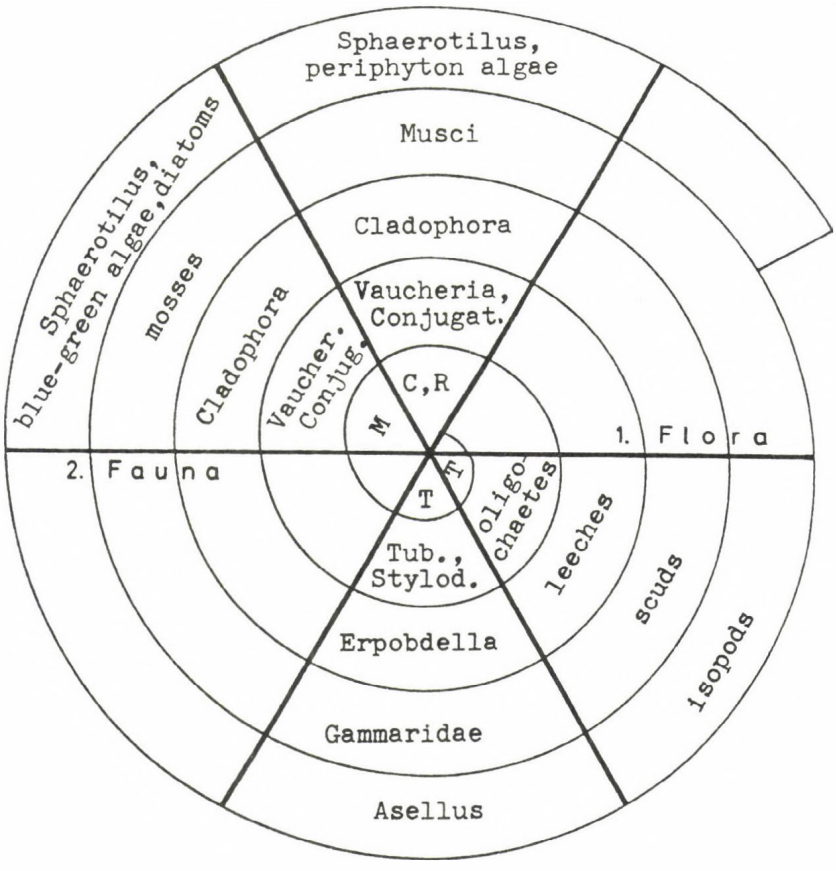

Fig. 1 Bioaccumulation spiral with empirical indicators for the heavy metal content of rivers. Separate representation for members of the aquatic flora and fauna. The indicator value increases towards the outside of the spiral.

C = Callitriche
R = Ranunculus
h.W. = submerged macrophytes
T = trichoptera
Tub. = tubificidae
Stylod. = Stylodrilus

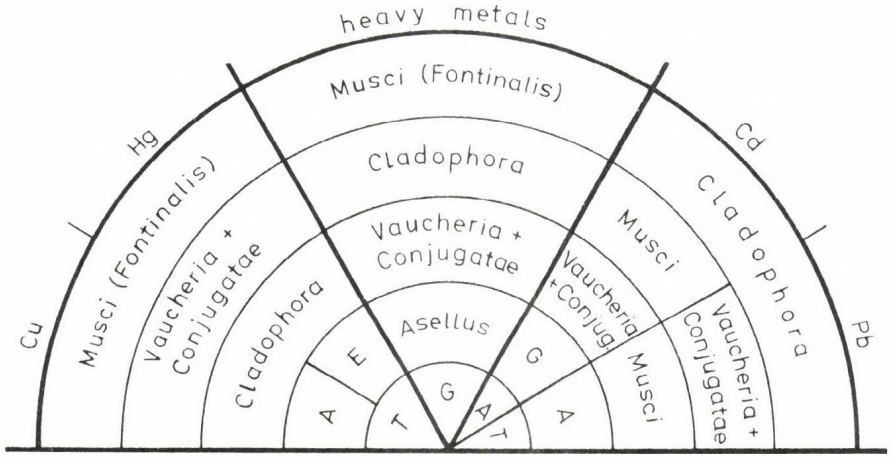

Fig. 2 Bioindication sectors with respect to individual heavy metals and to the sum of those metals. The indicator value increases towards the outside of the sectors with the best indicator in the outermost ring.

A = Asellus
E = Erpobdella
G = gammaridae
T = trichoptera

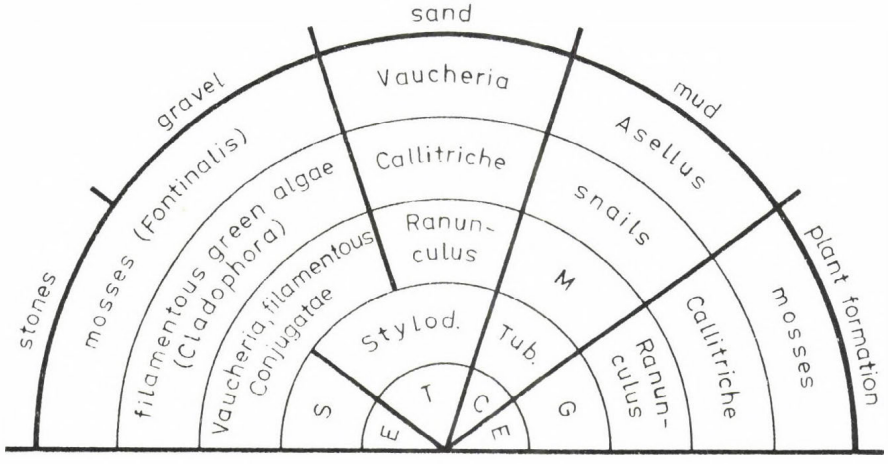

Fig. 3 Bioindication sectors with respect to different substrate types or ecosystems.

C = chironomidae
E = Erpobdella
G = gammaridae
M = mussels (Pisidium, Sphaerium)
S = snails
Stylod. = Stylodrilus
T = trichoptera
Tub. = tubificidae

Nevertheless, some of the rarer species theoretically have good potential as bioindicators, e.g., the waste water fungus Leptomitus, the mussels Sphaerium and Pisidium, and several types of snails.

The accumulation sectors in Fig. 2 relate to determinations of one or several metals in the biotope, whereas in Fig. 3 they relate to the substrate-specific biocoenoses in a biotope or ecosystem. In fluvial ecosystems, the indicator value of certain submerged plants may exceed that of the listed genera of the aquatic fauna. The musci, e.g., Fontinalis antipyretica, represent indicators with a very pronounced accumulation potential which occur widely and all year round.

Well-founded average values of bioconcentration are obtained by carrying out measurements over a period of 1 - 2 years with a sampling frequency commensurate with the temporal stability of metal contamination, but with at least one investigation per month. Results of the described sensitivity analyses provide, in contrast to the direct but problematical determination of mean metal concentrations in the water, an accurate and detailed picture of the long-term metal burden of a river ecosystem [4]. Systematic analyses of several aquatic bioindicators clearly reflect the heavy metal contamination level in the water body and can be used effectively to locate point emission sources. We are convinced that the ability of aquatic ecosystems to accommodate toxic substances cannot be estimated on the basis of emission limits alone, but that such an estimation must involve direct determinations of pollution levels in the receiving water body. The preventive protection of natural waters requires comprehensive knowledge of the distribution patterns of contaminants in the aquatic biota. Insights gained over the course of many years were translated here into element and substrate specific indicator schemes. These insights can be used to optimally plan and carry out future investigations which may become necessary in order to establish the ecological state of a water body or for legal reasons.

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DISCUSSION

SVOBODOVÁ, Z: The series of amounts concerning mercury in various organs of fishes very strongly depend on the quality of water. In which types of waters did you construct the series for organs of fishes concerning the amount of mercury?

WACHS, B: The here given series are based on measurements in rivers with an average metal load, i.e. the concentration in water is 0.03-0.1 µg/l Hg. If the level will be much more higher, then Hg is predominantly stored by the liver or the kidney.

SALÁNKI, J: 1/ Measuring heavy metal concentrations in various organs of predatory and non-predatory fishes of Lake Balaton we found differences in the order of metal concentrations depending on the fish species. My question is what type of fishes have you considered when speaking about "fish" in general?

2/ According to your results gills contain very low concentrations of heavy metals. We found, on the contrary, that gills have usually higher concentrations of metals than most of the other organs. Our data are calculated to dry weight, which can give different magnitude as if calculated to wet weight. Could you comment on and clarify this point?

WACHS, B: 1/ When I have spoken of fish from running waters in general I meant the more or less common fish populations of our rivers. In predatory fishes from rivers the heavy metal concentrations are usually not higher than in other fishes, they are at the same level or lower and don't follow the hypothesis of the food chain. The most values we have got from the following species: chub /Leuciscus cephalus/, Vimba bream /Vimba vimba/, pike /Esox lucius/, bream /Abramis brama/, and rainbow trants /Salmo gairdneri/. 2/ Usually the values of fish are calculated to wet weight. Before analyzing the gill book sample has to be prepared by cleaning it from organic material or other strange substances. In general we have found higher concentrations in liver, kidney and spleen than in gills, only in acute cases with suddenly high metal concentrations in the water flow could we measure high values in the gills due to their ability of fast adsorption. Discrepances are possibly due to the different ways of calculation.

V.-BALOGH, K.: In your results Hg concentration was higher, than Cd in fish. On the contrary it was found that the concentration of Cd was higher than the concentration of Hg.

WACHS, B: Our measurements are from averaged contaminated rivers or from rivers with normal background concentrations. If in your case the Cd level of water may be higher for example, then the sequence may be changed.

WEIS, P: The proportions of metals in sediments are not necessarily the same as the proportions in the fish living over those sediments. For example, we have a study site by a Hg dump. The Hg to Cd ratio is 10:1, but the fish muscle ratio is 1:3 suggesting that Cd is 30 times more available than Hg for biological uptake. This may relate to the lower solubility of HgS than of CdS.

WACHS, B: Well, this behaviour in the ecosystem is of great importance. For the biological uptake of heavy metals the metals emitted into the receiving waters should not change by forming insoluble compounds in the water body.

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HEAVY METAL AND RADIONUCLIDE TRANSFER AND TRANSPORT BY MARINE ORGANISMS

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ABSTRACT

All marine organisms accumulate metals and radionuclides to some degree and as a result redistribute these elements by various physiological mechanisms and through their ecological behaviour. Water, food and sediments are the three source terms from which marine biota acquire metals and radionuclides. Uptake from water can occur by passive surface adsorption, absorption across body surfaces, or a combination of both. Elements ingested with food are either absorbed across the gut and assimilated into tissue or they are excreted. The relative importance of the food and water pathway in uptake varies with the element and the conditions under which exposure occurs. Less is known about mechanisms of uptake from sediments, but most evidence suggests that, compared to water and food, bioavailability from sediments is relatively low. These dynamic processes are controlled by many environmental and intrinsic factors including exposure time, physical-chemical form of the pollutant, salinity, temperature, competitive effects with other elements, organism size, physiology, life cycle and feeding habits. Accumulated metals and radionuclides are transported actively by vertical and horizontal migrations of organisms and, more important, passively by release of biogenic particulates (fecal pellets, molts, etc.). As these particles sink, they release contaminants to deeper waters and sediments through remineralization processes, scavenge other elements from the water column and are ingested by meso- and bathypelagic species. Potential biological mechanisms for returning sediment-associated contaminants to the upper waters are also discussed.

INTRODUCTION

The ability of marine organisms to accumulate heavy metals and radionuclides is well documented; however, one aspect less well understood is how and to what degree they affect the distribution of these elements in the marine environment. Trace contaminant distributions strongly depend upon current and water mass movements, eddy diffusion and sedimentation processes. Movements of the contaminants associated with biota are also subject to physical and geological transport processes but, in addition, are affected by bioaccumulation, retention, and subsequent food chain transfer, horizontal and vertical migration of many species, and passive sinking of biodetritus. Clearly, the relative importance of these biological transport mechanisms compared to physical and chemical processes will be a function of the oceanic biomass at any given location. Many of the aspects of

bioaccumulation, bioavailability and metabolism have been the subject of several recent in-depth reviews (Davies, 1978; Bryan, 1979; Swartz and Lee, 1980; Lee and Swartz, 1980; Fowler, 1982; Luoma, 1983; Trabalka and Garten, 1983). The brief review which follows attempts to describe some of the more important biologically-mediated pollutant transfer and transport processes which occur from the time these substances enter and leave the surface layers of the water column until they ultimately reach the benthos. Environmental and physiological factors which control these processes are also discussed.

CONTAMINANT BIOACCUMULATION PROCESSES

Uptake from water

Uptake from water can occur by adsorption of the substance onto body surfaces or absorption across body surfaces such as gill and gut walls, or a combination of both. For heterotrophs the alternative mode of accumulation is through ingestion and assimilation of contaminated food or particulate matter. The actual mechanism by which trace metals traverse the cell membrane is thought to involve specific and non-specific carrier molecules (see Luoma, 1983). The relative ability of organisms to concentrate elements is often expressed by a concentration factor, defined as the ratio of the amount of element per unit fresh weight of tissue to that in an equal weight of sea water. Since these ratios take into account only the amounts of element in water and the organism, they give no information on the relative importance of the different routes of uptake. Furthermore concentration factors are not constant but vary considerably, since element body burdens are in a state of dynamic equilibrium and are the net result of both uptake and elimination processes occurring simultaneously. Depending on the element and the chemical species, concentration factors range from roughly 10^0 to 10^6. Concentration factors such as these attest to the organism's ability to concentrate elements from sea water. However, biological concentration processes become even more striking when it is considered that recent advances in sea water metal analyses (Bruland, 1983) have shown that certain metals are present in sea water at levels as much as 3 orders of magnitude lower than previously believed, indicating that many concentration factors may be underestimated by the same order of magnitude.

The variability in concentration factors is illustrated in Figs. 1 and 2. The uptake response in a given organism is highly dependent upon the element. For example, concentration factors in euphausiids can range from 10^2 for non-essential elements like plutonium to nearly 10^4 for highly lipophilic metal compounds such as methyl mercury (Fig. 1). Likewise, the same order of magnitude differences are evident for a given element accumulated by different marine organisms (Fig. 2). Variations are likely to be greater than the range shown in Fig. 2 since higher concentration factors are generally found in smaller species due to their greater relative surface area for adsorption (Fowler, 1982).

Phytoplankton, because of its large surface area to volume ratio, rapidly takes up radionuclides and metals and reaches extremely high concentration factors. Equilibration times are short (minutes to hours), and the initial process, thought to be passive for many elements, involves rapid sorption to the surface, perhaps by cation exchange, followed by slower diffusion across the cell membrane and binding within the cell (Davies, 1978; Fisher, et al., 1983a).

Likewise zooplankton attain high radionuclide and metal concentration factors following adsorption or absorption (Fig. 1). Uptake rates strongly depend on the element, with reported equilibration times ranging from several hours to several days. With crustacean zooplankton, the amount of

element taken up from water is controlled by molting due to the large fraction often adsorbed to the exoskeleton (Fowler, 1982).

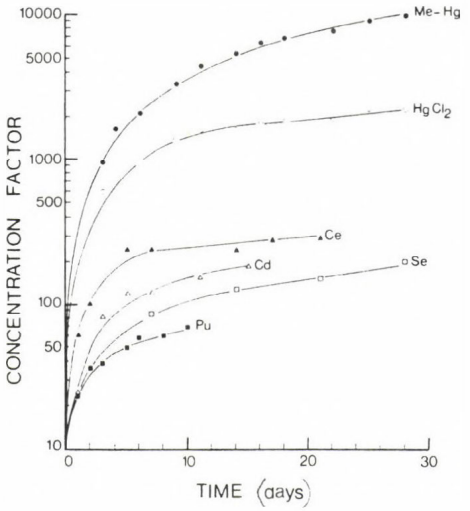

Fig. 1. Accumulation from water of various metals and radionuclides by euphausiids (from Fowler, 1982).

Macroinvertebrates, fish and sessile algae also absorb metals and radionuclides from water although the degree of uptake is usually less than that of smaller organisms since the role of surface area in total accumulation is of far lesser importance in larger species. Uptake is generally nonlinear and often biphasic with an initial rapid component representing surface adsorption followed by a slower rate of accumulation into internal tissues. Because in larger organisms internal tissues are often relatively isolated from the surrounding sea water, equilibrium times based on element absorption from water are normally longer (days to weeks).

Theimportance of the initial component of uptake depends to some extent on the surface characteristics of the organism. Hardshelled, calcareous animals may deposit much of the element in the shell during growth. Indeed substantial concentrations of radionuclides and metals are present in mollusc shells and exoskeletons of crustaceans (Fowler, 1982). Soft bodied organisms are able to equilibrate their internal tissues more rapidly. The surface mucous coating plays a primary role in the initial complexing of the element. For example, the epidermis of tuna, representing about 0.25% of the fish's weight, contains about 52% of the total lead content (Chow et al., 1984). The degree of pollutant build-up in tissues depends on the chemistry of the element, the number of binding sites, potential for detoxification, retention time in a tissue, sexual cycle, and general physiology of the organism. Although not always, liver and kidney of both invertebrates and vertebrates often contain the highest concentrations of metals and radionuclides. Muscle normally concentrates inorganic contaminants to a much lesser extent except, for example, in the case of methylmercury (Pentreath, 1976) and As (Fowler and Unlü, 1978).

The chemical species and oxidation state of elements also greatly affect uptake. For example, RuCl is significantly more bioavailable to

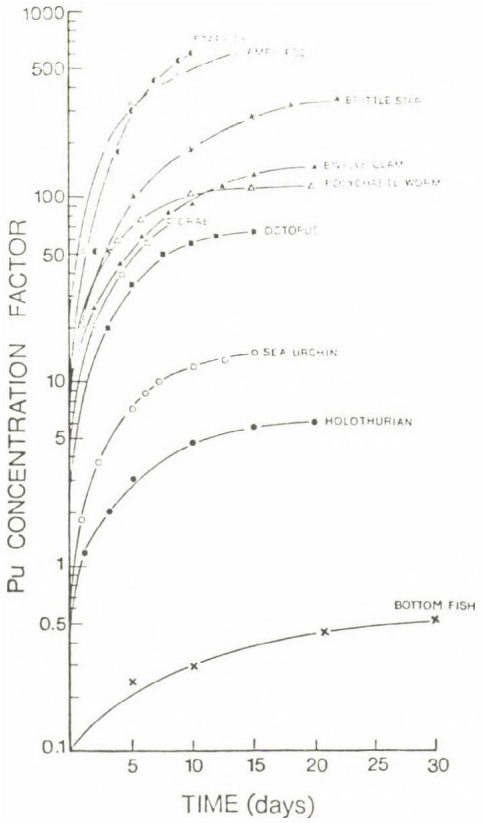

Fig. 2. Concentration factors for plutonium uptake from water by benthic organisms (from Fowler and Guary, unpublished results).

benthic species than nitrato-nitrosyl complexes of ruthenium (Keckes et al., 1967) and the oxidized form of technetium (Tc + 7) is taken up to a greater degree by worms than the reduced forms (Tc + 4) (Fowler et al., 1981). It has also been shown that iron hydroxide particles sorb to surfaces of phytoplankton, a mechanism which may enhance the uptake of other elements since metal hydroxides are effective scavengers of elements (Davies, 1978). Reduction in uptake has been noted for elements complexed with organics; in fact, there is considerable evidence that in many organisms only ionic species or possibly only the free ions are bioavailable (see Davies, 1978; Luoma, 1983). An example of the relative bioavailability of methyl and inorganic mercury is shown in Fig. 1.

Element uptake in many species is proportional, or nearly proportional to the concentration of the element in sea water. Some species show little or no increase in element concentration in response to elevated levels in their surroundings. This may be a net result of physiological processes (uptake, storage, and elimination) or simply that uptake is reduced by saturation of binding sites on or within the organism. However, there is strong evidence that for essential metals like Zn, crabs, polychaetes, and fish can effectively regulate their tissue content (Luoma, 1983).

Generally speaking, metals and radionuclide uptake rates correlate positively with temperature in a variety of species, including phytoplankton, zooplankton, benthic crustaceans, mussels, and fish (see Bryan, 1979; Fowler, 1982; Luoma, 1983). However, there are some notable exceptions with crustaceans (Fowler and Unlü, 1978) and bivalves (Phillips, 1980), indicating that temperature has little or no effect. In the case of crustaceans, one explanation is that at elevated temperatures crustaceans molt more frequently and thus eliminate more rapidly the accumulated element. Hence, the net effect is lower levels than those in animals exposed at lower temperatures.

Element uptake rates in marine species generally show an inverse correlation with salinity (Fowler, 1982). This effect, usually attributed to lesser amounts of competing ions in low salinity waters, is thus most pronounced in estuaries and coastal waters receiving runoff.

The coexistence of several inorganic elements in sea water can also affect element accumulation. Studies on this aspect are few, and Phillips (1980) points out that uptake responses are highly variable and largely dependent on the combinations and relative concentrations of the elements involved.

The pH of sea water may also affect element absorption to some degree. Although of little or no consequence in open ocean waters where the range is extremely narrow, pH may come into play in estuaries where mixing with fresh water takes place.

Uptake from food

Absorption and tissue distribution of ingested metals and radionuclides depend on the bioavailability of the element, which in turn is a function of the type and degree of ligand binding within the food matrix (Luoma, 1983). Biologically essential elements such as Zn, Fe, Co, Mo are absorbed across the gut with relatively high assimilation efficiencies; however, non-essential elements or those which are associated with particulates like Ru, Ce, Pu, Am are poorly absorbed and are excreted with the feces (Pentreath, 1977; Fowler, 1982). Element absorption through the gut can lead to tissue distributions of the element which differ considerably from those achieved by uptake from water (Guary et al., 1982). The liver or hepatopancreas is often the principal organ of accumulation and the distribution of an ingested element in tissues has been shown to depend on species, food quality, chemical form of the element, and the time elapsed following ingestion. The build-up of ingested elements in tissues also depends on the rate of elimination. When excretion is slower than absorption, accumulation will increase with repetitive ingestion of contaminated food.

Elimination

Element uptake is not an irreversible process. Loss can occur by passive desorption, active excretion of soluble element, and particulate excretion such as feces, molts and reproductive products. Most often metals and radionuclides are lost from marine species more slowly than they are accumulated. In the absence of the contaminant, loss is rapid at first due to desorption of loosely-bound element or defecation; at a later stage a much slower loss rate is observed as a result of release from stronger binding sites within the animal. The biological half-life ($T_{b1/2}$), or time to lose 50% of the radionuclide, is an often-used parameter to quantify loss. Loss rates are rarely constant; hence, there are $T_{b1/2}$ characteristic of the various individual radionuclide pools within the animal. Biological half-lives vary from several hours or a few days for phytoplankton and zooplankton (Fowler, 1982; Fisher et al., 1983a,b) to

several days or months for larger species (Pentreath, 1977; Bryan, 1979; Phillips, 1980; Fowler, 1982); nevertheless this parameter is highly element dependent. Other factors that can affect loss rates are temperature (not really a variable in the deep sea) and the time that the organism was exposed to the metal or radionuclide. Furthermore, loss will vary greatly with species; for example, molting crustaceans, particularly small forms, lose a large fraction of their element body burden at fairly frequent intervals (Fowler, 1982). Molting rates increase with temperature, therefore element elimination via molting may be important in warmer surface waters. We know little about molting rates in the cold, deep sea but the crustaceans are relatively large and likely have long inter-molt periods.

Another important excretion route is defecation. Particularly for non-biologically essential elements or those which are poorly assimilated, excretion via feces becomes increasingly important. Preferential assimilation of organics over the ingested radionuclide often leaves the latter in a more concentrated state (unit weight basis) in fecal material as it passes through the animal. There are several studies which underscore the relative importance of defecation in eliminating heavy metals and radionuclides from marine species (Pentreath, 1977; Fowler, 1982; Hardy et al., 1984). A typical example is illustrated in Table 1.

Table 1. Relative distribution of element efflux through euphausiids by molting, defecation, and soluble excretion (after Fowler, 1982).

Element	Molting (%)	Defecation (%)	Soluble excretion (%)
Zn	1.1	92.6	6.3
Cd	3.3	84.5	12.2
Se	2.4	54.4	43.2
Hg (inorg.)	2.5	29.1	68.4
$^{239,240}Pu$	0.8	98.6	0.6

Data concerning transfer of elements into reproductive products and larva are limited. Nevertheless, there is evidence from laboratory studies (Woodhead, 1970) to show that eggs readily accumulate long-lived radionuclides. Clearly it is probable that excretion could occur once the radionuclide has been transferred from reproductive tissue into eggs.

Relative importance of food and water vectors

There is much controversy in the literature as to whether food or water is the more important uptake vector for metals and radionuclides. The reader is referred to Pentreath (1977), Fowler (1982) and Luoma (1983) for critical reviews on the subject. Obviously, many biological, chemical and environmental factors act in concert to regulate the relative importance of the two pathways. However, two basic conditions primarily govern which uptake pathway will predominate: (1) the relative concentrations of the contaminant in water and food and (2) the relative abundance of food biomass available for ingestion. Considering both the non-homogeneous distribution of organisms in the sea and the wide range of concentration factors for different metals and radionuclides, it is highly probable that no one pathway always predominates but that the relative contribution of each route depends upon the prevailing ecological conditions and, thus, varies in time and space. Because of the inherent difficulties in delineating these pathways,

few studies have addressed this question in a rigorous manner. Most evidence has been derived from either indirect estimates based on theoretical calculations or short-term experiments carried out under ideal feeding conditions; hence, our ability to extrapolate these findings to the natural environment is limited.

For many invertebrates, especially the small planktonic forms, uptake from water often predominates. This has been noted for Cd in copepods (Sick and Baptist, 1979) and several radionuclides in euphausiids (Fowler, 1982; Fisher et al., 1983b). In contrast other studies with euphausiids have shown the importance of food in the long-term accumulation of Zn, Cd and Se (see Fowler, 1982).

Renfro et al. (1975) allowed shrimp, crabs, and small fish to accumulate ^{65}Zn for 3 months from water or from a combined food and water pathway. Despite continuous ingestion of radioactive food, shrimp and crabs in both treatments reached similar ^{65}Zn levels. In contrast, for fish the food pathway accounted for 2.5 times more ^{65}Zn than that obtained from water alone. A comparison of stable and radioactive Zn concentration factors indicated a lack of isotopic equilibrium after 3 months and suggested Zn pools within the adult which exchange slowly, if at all, with Zn taken up from water or food. Similar conclusions have been drawn from studies with fish. Only in the case of monovalent ^{137}Cs and inorganic Hg does it appear that the water input into fish is significant (~50%)(Pentreath, 1977).

Certainly element input through food is important, and food chain transfer is enhanced by: (1) high concentration factors in prey, (2) high assimilation efficiencies in predator, and (3) strong retention of the assimilated fraction by predator.

Food chain transfer and biomagnification

Elements are often visualized as moving along food chains from prey to predator with the concentration increasing or decreasing at each trophic level. The basic assumption is that element input comes only from food, and that biomagnification, or increase up the food chain, would occur if the element was assimilated and retained. However, as discussed above food is not the sole vector for element input. Most evidence for or against the biomagnification hypothesis comes from direct comparisons of element concentrations in whole organisms from different trophic levels. In reality the conclusions drawn depend on which tissues are compared. Where such comparisons have been made, higher concentrations have normally been found in the prey than in the predator (Pentreath, 1977; Fowler, 1982; Luoma, 1983).

Plutonium biomagnification has been proposed to occur in the mussel-starfish food chain; however, recent studies (Guary et al., 1982) have revealed that starfish readily accumulate Pu from water and that the resultant tissue distribution following uptake from water closely resembles the Pu tissue distribution in starfish contaminated in the environment. Only for methyl-Hg (Pentreath, 1977) and ^{137}Cs (Pentreath, 1977; Thomann, 1981) in top level fish is their strong evidence for food chain biomagnification.

CONTAMINANT TRANSPORT

In the following discussion biological transport pathways (Fig. 3) are examined with respect to their importance in the biogeochemical cycles of metals and radionuclides.

Horizontal and Vertical Migration

Large mobile organisms like fish moving through polluted waters can transport contaminants away from their source. Horizontal transport is most important for elements with long $T_{b1/2}$; however, to what extent this process is effective has been little studied. Contaminants which associate

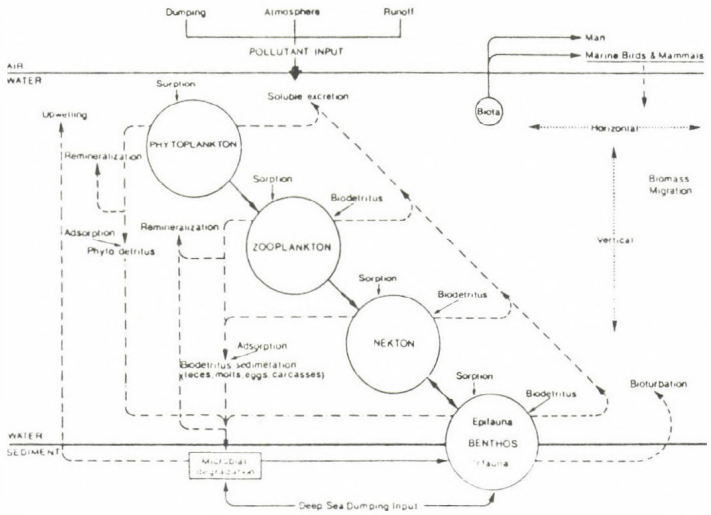

Fig. 3. Element transfer and transport by biological processes (Fowler, 1982).

with sediments and benthic organisms are, in the short term, probably little affected by horizontal migration. Aarkrog (1977) found Pu-contaminated biota and sediments 15 km from the Pu source a few months after an accident. Two and 6 years later the radius of biological contamination had spread to approximately 30 and 40 km, respectively. These timescales and distances indicate a slow dispersion of sediment-bound Pu by marine organisms. In contrast, bioavailable elements in the aqueous phase may be more widely dispersed by moving organisms. For example, high concentrations of cadmium (100 to 200 ug g^{-1} dry) have been found in the epineustonic seaskater, Halobates, which lives on the contaminant-enriched surface microlayer (Cheng et al., 1976). It is conceivable that many wind-driven epineustonic forms (insects, Sargassum weed, jellyfish, etc.) could transport pollutants over considerable distances, however, it is doubtful that biological transport can compete with large-scale horizontal advective processes, except perhaps under a unique set of conditions.

Many pelagic species undertake diel vertical migrations. During these migrations some organisms are eaten and thus contaminants enter the food chain and at the same time move vertically. Organisms that are not eaten move across physical boundaries (pycnocline, thermocline, etc.) and can presumably excrete or accumulate metals and radionuclides in a different water mass. Pearcy and Osterberg (1967) collected migrators from different depths in a contaminated area of the North Pacific, and based on ^{65}Zn concentrations in migrants and nonmigrants in the 0- to 1000-m water column, they estimated that roughly 40% of the ^{65}Zn in organisms sampled was transported twice daily through the base of the pycnocline at 150 m. Euphausiids, the principal migrators in this region, contained relatively high levels of ^{65}Zn throughout the year, leading Osterberg et al. (1964) to suggest that biological transport of Zn across the pycnocline by these organisms was more important than by physical processes. They demonstrated that because of vertical migration, a major loss of ^{65}Zn from the mixed layer would be caused by predation on euphausiids below the pycnocline.

Kuenzler (1969) studied biological transport of radionuclides in the eastern Pacific and found that only about one-fourth of the zooplankton biomass in the top 500 m migrated out of the mixed layer carrying down and eliminating cobalt below the thermocline. Under these conditions the quantity of cobalt in the top 100 m would be removed in about 56 years, a turnover time about the same as that computed for vertical eddy diffusion. His work was the first to recognize the importance of biogenic particulates in effecting vertical transport.

Large Particle Transport

While vertical migration is an important transport process in the upper waters, it cannot account for the presence of contaminants in organisms at great depth. Osterberg et al. (1963) noted differences in the short half-lived ^{95}Zr-^{95}Nb pair in deep and shallow sea cucumbers which indicated a transit time of 7-12 days to 2800m. This implied a sinking rate of 230-400 m day^{-1} and the authors proposed that zooplankton fecal pellets were transporting the radionuclides downward. More recent work has shown that large biogenic particles are often extremely rich in heavy metals and radionuclides. Concentrations of many trace elements are often higher in biogenic debris than in the organisms producing them (Table 2). Since most biogenic debris is produced in the rich surface layers, these enriched particles will be instrumental in removing contaminants from upper waters. As biogenic particles sink, they can further scavenge metals and radionuclides from the water or release elements back to the water as the particles decompose. In this respect biodetritus has a profound effect on element distribution in the sea (Bruland, 1983). In fact, based on these data and mass balance considerations of river input and sediment output, some studies have concluded that the settling of fecal pellets and aggregates is the most important mechanism effecting the vertical transport of many trace elements (Lowman et al., 1971; Cherry et al., 1978; Li, 1981).

Evidence for fecal pellet control on element distributions in the sea comes from several different studies. Large volume filtration and sediment

Table 2. Environmental levels of trace elements and radionuclides in macrozooplankton, their particulate products and their natural food (microplankton). (Data from Fowler, 1982 and Higgo et al., 1980).

	(ppm dry)			
Element	Macroplankton	Fecal Pellets	Molts (%)**	Food (microplankton)
Ag	0.7	2.1	2.9 (31)	0.7
Cd	0.7	9.6	2.1 (22)	2.1
Co	0.2	3.5	0.8 (34)	0.9
Cr	0.9	38	5.3 (48)	4.9
Cu	48	226	35 (6)	39
Fe	64	24000	232 (28)	570
Mn	4	243	12 (21)	18
Ni	0.7	20	6.7 (78)	8.1
Pb	1	34	22 (100)	11
Zn	62	950	146 (18)	483
Hg	0.3	0.4	0.2 (4)	0.1
Se	4	7	2 (3)	3
^{239}Pu*	0.4	98	4.8 (90)	4.0
^{210}Po*	1100	24500	360 (2.5)	3400
^{232}Th*	0.35	250	2.6 (57)	17
^{238}U*	21	520	245 (90)	340

* pCi/kg dry
** Percent of total body burden contained in molt.

trap experiments have shown conclusively that fecal pellets and fecal matter are responsible for the bulk of the vertical mass flux in the upper waters of the ocean (Bishop et al., 1978; Spencer et al., 1978, and others). Analyses of freshly released zooplankton particulates products (e.g. Table 2) coupled with radiotracer studies have been used to model the role these particles play in the vertical transport of several elements (Higgo et al., 1980; Fisher et al., 1983b; Gorsky et al., 1984). Recently measurements of metals and radionuclides in sediment trap samples have allowed directly assessing the flux of these elements associated with biogenic detritus throughout the water column (Spencer et al., 1978; Brewer et al., 1980; Livingston and Anderson, 1983; Fowler et al., 1983). In one such study in the upper few hundred meters in the northeast Pacific, Fowler et al. (1983) found that transuranic (Pu and Am) concentrations in large particles (principally fecal pellets) and transuranic flux increased with depth to 750m, suggesting active scavenging of the radionuclides through these strata. Americium was scavenged to a greater degree than Pu as evidenced by increasing Am:Pu ratios with depth. Transuranics were accumulated by an observed phytoplankton bloom at the surface, repackaged by zooplankton grazing and rapidly removed to depth by sinking fecal pellets. Flux-derived particulate residence times for two depth intervals in the upper mixed layers (0-80m and 0-250m layers) were computed to be 2 and 13 years for $^{239+240}$Pu and 1.5 and 4.5 years for ^{241}Am. However, fecal pellets may not maintain their enrichment of elements at depth. For example aged fecal pellets from 5000m contained 1 to 2 orders of magnitude less Zn than those recently produced in surface waters (Spencer et al., 1978; Fowler, 1982). There is also evidence from deep water sediment trap experiments that Pu and Am are regenerated from particulate matter near the bottom (Livingston and Anderson, 1983). Table 3 gives the half-times for release of several elements from typical debris; these elements differ greatly in their reactivities, however, the element $T_{b1/2}$'s in decomposing biodetritus only vary within approximately an order of magnitude. Furthermore fecal pellets retain the elements to a greater degree than either molts or carcasses principally because the latter forms decompose more rapidly than fecal pellets. This indicates that of the biodetrital forms, fecal pellets have the greatest potential to transport trace elements downward.

Table 3. Remineralization half-times (days) for elements in biogenic particles. Temp.= 13°C. (Data from Fowler, 1982; Fisher et al., 1983b).

Element	Fecal Pellets	Molts	Carcasses
Zn	2.0	1.7	7.8
Hg (Inorg.)	14.1	2.3	3.1
" (methyl)	6.0	4.1	2.6
Se	3.9	-	-
$^{239+240}$Pu +VI	9.7	4.7	5.3
" " +IV	23.0	-	-
^{241}Am	41.0	3.0	-
^{144}Ce	7.5	10.6	17.3
^{210}Po	3.5	-	-

Benthic Boundary Layer Processes

Not all inorganic contaminants associated with particles are recycled to the water and many eventually become incorporated in the sediments. As

contaminated sediments may be a source for pollutants rather than a sink, biological processes that remobilize, transfer and transport sediment-associated metals and radionuclides in both shallow and deep-sea areas have been examined (Fowler, 1982).

At the sediment boundary where levels will be the highest initially, bacteria and other meiofauna can accumulate these contaminants. Bacteria may resolubilize elements in different forms or create metal precipitation by forming a reducing environment. Sediment-associated microorganisms are thought to be responsible for the methylation of Hg although its rate of formation appears to be slow. Unfortunately meiofauna have been overlooked with respect to contaminant transport, however, their relative importance in terms of biomass increases in the deep-sea.

Macrofauna can accumulate sediment-associated contaminants from pore water, by filtering resuspended sediment, or by ingesting only the surface layers of sediment. These processes account for the presence of radionuclides in deep-sea holothurians (Osterberg et al., 1963). Laboratory studies have shown that uptake of most radionuclides from sediment is very small and is highly dependent upon sediment type (Fowler, 1982; Vangenechten et al., 1983). This is evident from the data in Table 4 which show that compared to water, the transfer of metals from sediment is low. For Pu and Am, the majority of the radionuclide in worms is taken up from pore water (see Fowler, 1982).

Table 4. Transfer factors for uptake of radionuclides from sediments by Nereis. Numbers in parentheses are concentration factors based on uptake from water (from Fowler, 1982).

Uptake (Days)	C.F. or T.F.							
	$^{239+240}Pu$	^{241}Am	^{55}Fe	^{95}Zr-^{95}Nb	^{137}Cs	^{106}Ru	^{60}Co	^{115}Cd
40	0.0014	0.0005						
(15)	(200)							
88			0.019					
11				0.01	0.2	0.006	0.06	
(11)				(4)	(6)	(6)	(6)	
8								0.12
(12)								(22)

Sediment reworking activities by mechanical action, ingestion and egestion are of prime importance in the redistribution of elements in the benthic boundary layer. Dyal et al. (1979), using computed mixing rates of 1 cm^2y^{-1}, determined that at a 2800m dumpsite in the Atlantic about 0.3% of the sediment-associated ^{137}Cs could have been released to overlying waters by bioturbative processes. Frequent observations of disturbed radionuclide profiles at depth in intact sediment cores also imply high biological activity in the sediments.

Biodeposition or defecation is an important process in that some benthic invertebrates produce several Kgs of feces per year. In a contaminated environment fecal casts of infauna and epifauna, enriched in certain elements, would be available as food for other organisms. Coprophagy is widespread in the marine environment, but we lack information on transfer coefficients for organisms ingesting this material. Probably the best information on sediment-bound contaminant migration comes from studies at Thule, Greenland where in 1968 PuO was accidentally deposited as a point source in sediments at 200m depth (Aarkrog, 1977). The surveys carried out in 1968, 1970 and 1974 showed that by 1970 contamination levels in organisms had decreased by an order of magnitude but since that time the decrease has

been much less. The half-distance for decrease in Pu concentration from the point source was 3 km in sediments but 5-6 km for certain marine organisms. Furthermore, lesser decreases with distance in starfish and shrimp compared to sedentary molluscs indicated the greater mobility of the former animals. Even in this high energy environment, greater than 99% of the computed Pu inventory still remained bound to sediments. Furthermore, no radioactivity from the accident was found in overlying surface sea water, fish, zooplankton, sea plants, sea birds or marine mammals. These findings are of importance when considering use of deep-sea sediments as burial sites for radionuclides and sludges containing heavy metals. The deep sea is a relatively low energy environment and the biomass there is much less than that encountered at Thule; thus, horizontal migration of such elements would proceed at a much slower rate

More difficult to assess is the potential for sediment-associated metals and radionuclides to migrate vertically via the biota. Models examining this aspect (e.g. Lowman et al., 1971; GESAMP, 1983) have shown that concentration factors in plankton are not high enough to compete with upward physical transport processes. It is conceivable that during their diel ascent, abyssopelagic organisms (and/or their fecal pellets, molts, etc.) could be eaten, however, the magnitude of this pathway is expected to be small (Rice, 1978). In any case the food chain in this sort of "overlapping feeding ladder" would be long and an attenuation in trace element concentrations would be seen at each link because of fractional assimilation by succeeding organisms in the food chain. Only a "short circuit" in the food chain (e.g. man eating deep sea squid or fish) could result in transfer of initial levels of contaminants directly to top level predators.

Benthic larvae may be the one biological agent which would disperse contaminants over the greatest vertical distance. Many benthic fish and molluscs have larvae which have been caught in surface waters; thus, contaminants at depth could be directly transferred to epipelagic food chains.

Another biological transport mechanism being considered is the "rising particle hypothesis" (Yayanos and Nevenzel, 1978). This model envisages the rapid ascent of contaminated, lipid-rich particles following their release at depth by intense feeding activities and breakdown of organic matter. Calculations based on size and density of particles isolated from deep-sea amphipods show that such particles released at 5000m could reach the surface within one week to a year.

These mechanisms are only speculative since there are no quantitative data with which to judge their relative effectiveness in dispersing contaminants upwards. However, based on present knowledge it is thought that, on a macroscale, biological processes are extremely unimportant in dispersing contaminants compared to advective transport such as upwelling (GESAMP, 1983).

CONCLUSIONS

A general picture is now emerging about how organisms interact in marine biogeochemical cycles of metals and radionuclides, however, there are several aspects which remain to be clarified. One need for future research is to determine the relative importance of the food and water pathways in contaminant uptake. Another is to measure assimilation efficiencies for various elements in different foodstuffs. These are two key pieces of information for biotransport models.

The transport mechanism of greatest consequence is the release and rapid settling of contaminant-enriched biogenic debris. These materials are instrumental in redistributing surface-introduced metals and radionuclides throughout the water column, yet quantitative information on scavenging rates remineralization rates and vertical fluxes is still sparse. Continued use of sediment traps in conjunction with measurements on specific biogenic particles

like fecal pellets is recommended.

Sediments will be the major recipient of anthropogenic contaminants both through natural sedimentation processes and intentional dumping. Elements have been measured in many species living in deep-sea sediments, but owing to our general ignorance about deep-sea biological processes, we have no knowledge of pollutant flux through populations at depth. More quantitative biological studies in the deep ocean are needed before we can accurately estimate the degree to which deep-sea biota remobilize and return pollutants towards the surface.

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DISCUSSION

AMIARD-TRIQUET, C: I agree with you that the importance of biomagnification has often been exaggerated. But several field and experimental studies seem to indicate a biomagnifiaction of radioactive caesium. What do you think about this eventual biomagnification of caesium?

FOWLER, S.V: Yes, you are correct. I failed to mention that example in my presentation. There is evidence of this process occurring based primarily on levels in fish muscle.

V.-BALOGH, K: Biomagnification may occur for certain organic and inorganic metal compounds for example organic and inorganic Hg. What are the most important differences for example, which are the highest trophic levels which show this effect?

FOWLER, S.V: There are numerous examples which show higher Hg levels in top predators such as fish like tuna, swordfish, etc. Where examined, usually the methyl Hg fraction increases in the fishes' food chain and is believed to be the cause of the observed increase of total Hg concentration in

FOWLER, S.V: /cont./ the organisms, tissues as one proceeds along the food chain from secondary to tertiary consumer. It should be noted that the same phenomenon has not been observed in lower trophic level species /i.e. phytoplankton to zooplankton/ of the marine food chain.

LORCH, D: Is there any indication of isotopic discrimination between stable and radioactive isotopes by organisms during metal accumulation? This would have serious implications for tracer studies.

FOWLER, S.V: To my knowledge there have only been reports of this between ^{238}Pu and $^{239,240}Pu$ and between certain radioisotopes of cobalt occurring under field conditions. However these hypotheses have been tested under controlled conditions and have not been substantiated. Usually it is a result of poor chemistry on the part of the researcher, or poor counting statistics of low environmental concentrations of the radio nuclides in the samples.

SALÁNKI, J: The release of pollutants through physiological processes /molting, faeces, excretion, granule production/ may be the main pathway; however, there must also be a continual redistribution of these substances inside the animal to promote depuration from tissues and organs which accumulate and firmly bind metals and radionuclides. Is there any information about the rate of such redistribution in various animals?

FOWLER, S.V: I know of no such data on rates of transformation within tissues other than heavy metal and radionuclide depuration rates from individual tissues. For certain bone-sealsing elements ingested with food, there is normally a transfer from liver to bone or hard calcified tissue during the growth process. There are other examples of inter-organ transfer of elements but rate data are generally lacking.

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A COMBINED EFFECT OF MERCURY AND CADMIUM ON PLANKTON *IN SITU*

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Plankton and various toxicants occur everywhere, in all the fresh and marine waters on the earth. As these components are found together in natural water bodies, it is very important to trace their relationship.

At present our knowledge on the toxicity of metals is almost completely based on laboratory experiments. G.W.Bryan (1984) considers that many experiments were carried out under such unreal concentrations that the significance of results can be doubted. The summarizing article by E.Walsh (1983) on the effect of toxicants on plankton shows that particularly numerous works are devoted to the study of various metal effects on phytoplankton while that on zooplankton is considerably less studied not to speak about bacterioplankton and unicellulars. However, laboratory experiments are needed and they give a vast and useful information, extrapolation of these results under field conditions at present seem impossible. The conditions of laboratory investigations differ from those carried out under natural ones. To fill in the gap between laboratory experiments and observations in nature experiments in controlled aquatic ecosystems are necessary. In isolated controlled ecosystems of various complicity depending on the number of trophic levels investigations were carried out in the following directions (Parson T.R.1981;1982; Banse K.1982; Grice G.D., Rewe M.R.1982): the study of fundamental problems on ecosystem functioning and solving of applied tasks connected with pollution and aquaculture.

The majority of published investigations consider biological effect of one of the pollutants (Kremling K. et al.1978; Kuiper J.1982). Under real pollution in natural ecosystems several toxicants are always present and affecting. Therefore the Laboratory Marine Biology of the Latvian SSR Academy of Sciences carried out complex investigations on combined activity of mercury and cadmium on unicellulars, zoo-, bacterio-, and phytoplankton, as well as on the pigment composition of the latter in the Gulf of Riga in experimental ecosystems.

The most effective way to study a combined effect of several environmental factors is to apply a mathematical planning of factor experiment (Maksimov V.N.1980). The

methodical part of the experiment has been presented in our earlier publications (Andrushaitis A.G. et al.1984; Andrushaitis A.G.1984). Nine experimental bags were taken simultaneously with a single adding of mercury ($HgCl_2$) and cadmium ($CdCl_2 \cdot 2.5H_2O$). The metals were added to the experimental bags next day after filling.

Zooplankton

During the whole experimental period of 14 days there are no great changes in the total number of zooplankton in control and they are similar to those in the open sea (Fig.1).

Several hours after the adding of mercury and cadmium almost in all the experimental bags great changes in number of zooplankton occurred in comparison with the control. Concentration of mercury 10μg/l was an exception where the total amount of zooplankton decreased gradually reaching minimum on the 8th day, after that a stimulation was observed (the quantity of zooplankton on the 14th day was 9 times larger than that of the 8th day). At mercury concentration of 50μg/l a stimulation was observed already several hours after mercury adding, but during the experiment a depression of zooplankton reproduction was observed as to the control.

At cadmium concentration of 10 and 50μg/l in the first hours of experiment a short clearly expressed stimulation was observed in the reproduction of zooplankton as to the control. But further on during the experiment the number of zooplankton was either the same or lower than that of the control.

At 10μg/l of mercury and cadmium an inhibited reproduction of zooplankton was observed some hours after the adding of metals, however on the 4th day its stimulation was observed. The total amount of zooplankton decreased 10 times in between the 4th and the 8th day, but at the end of the experiment it was equal with that of the control.

At mercury concentration 10μg/l and cadmium 50μg/l several hours after the adding of metals the total amount of zooplankton decreased twice remaining on the same level till the 4th day, and then a further decrease followed and on the 8th day the zooplankton quantity is twice less than that in the control. After that an increase followed.

At mercury concentration 50μg/l and cadmium 10μg/l some hours after the adding of metals the amount of zooplankton did not change, only on the 4th day it decreased twice and a half in comparison with the beginning of the experiment. Starting with the 8th day a strong inhibition of zooplankton reproduction was observed.

In the bag with mercury and cadmium concentration 50μg/l for several hours after the adding of metals a slight stimulation was observed in the reproduction of zooplankton, but on the 4th day the total amount of zooplankton started to fall. It differed little from that in the control bag. Unfortunately due to technical reasons we could not follow the further changes of zooplankton quantity under this version. The basic amount of the total zooplankton is represented by Copepoda species Eurytemora and Acartia.

When studying the combined effect of mercury and cadmium

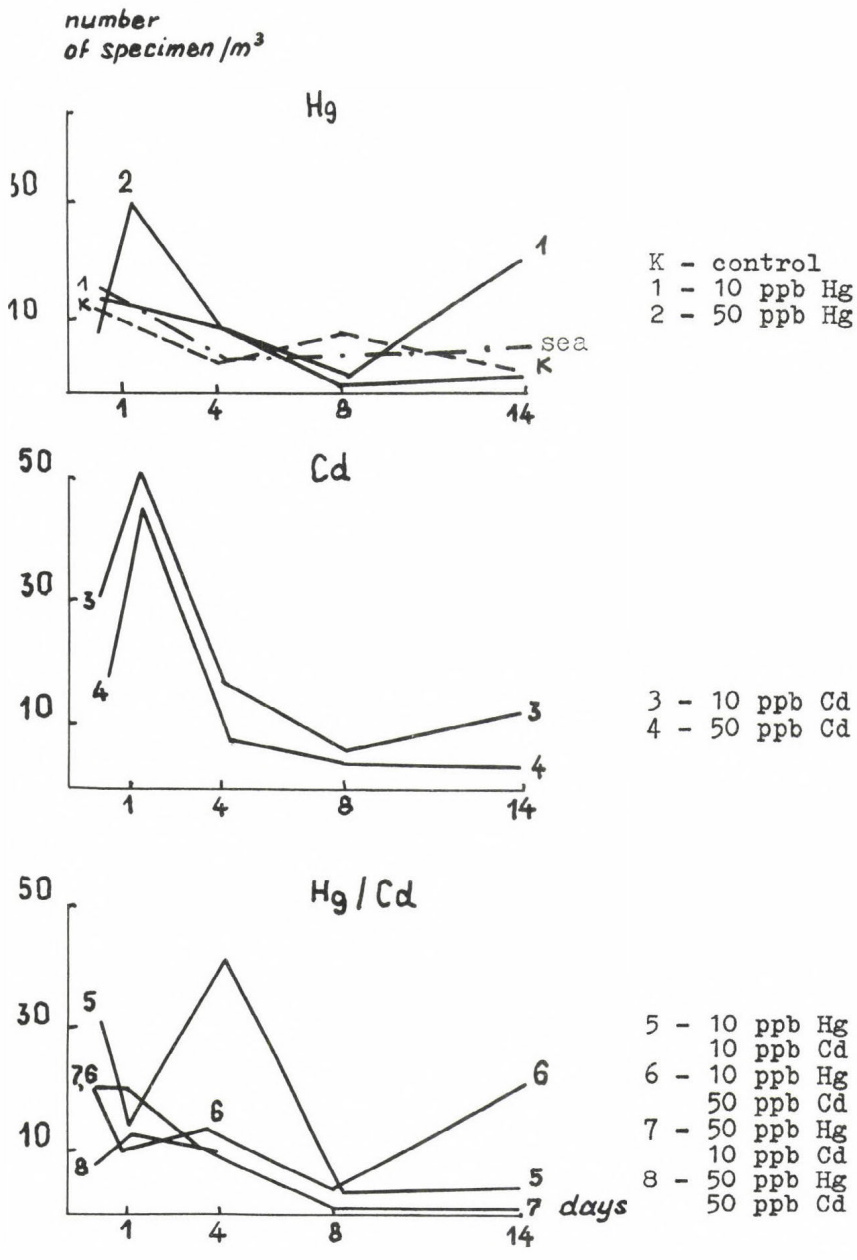


Fig. 1. Effect of mercury and cadmium on the total number of zooplankton.

a domination of copepods Eurytemora sp. of various age was observed in the control and the experimental bags of different metal concentrations from the beginning of the experiment to the 4th day. On the 8th day Eurytemora was still dominating in the control, but in bags with separate mercury and cadmium concentrations this copepod prevailed alongside with Acartia sp. At the combined effect of mercury and cadmium only the nauplia of Acartia sp. were observed. For experimental bags of different metal concentrations the 8th day appeared to be a transition point to another species domination. In the control that occurs only on the 14th day.

Unicellulars

According to the general basic principles in toxicology several zones of toxicological effect are distinguished: the area of acute toxicity (lethal), sublethal and stimulating concentrations in comparison with the control. We shall consider the results of heavy metal effect on protozoans in the given aspect (Fig.2).

High toxicity of mercury was observed already in the first day of experiment beginning with the concentration of 10 μg/l. But from the 9th day on a strong stimulation was noted. Possibly it is not a direct effect of mercury, but the absence of protozoan consuming while these concentrations of mercury destructed the higher consumers (zooplankton) and separate individuals of protozoa reached density above the control level due to high reproduction rate. Separately the effect of cadmium appeared to be less toxic than that of mercury. Unicellulars respond to the combined effect of mercury and cadmium in the following way: during the first hours of experiment "lethal effect" is observed in 4 bags (in samples protozoans were not stated) and only 3% (as to the control) at the ratio of metals 10/10 μg/l. High toxicity of mercury was also proved by the following example: the ratio of mercury and cadmium 1:5 was far less toxic than 5:1 (50/10 μg/l) during the whole observation period.

However, it should be marked that the heavy metals causing a certain stress situation in separate periods were not highly toxical for the protozoan community in general.

Phytoplankton

During the first 8 days of experiment the number of phytoplankton cells was observed to be falling under both concentrations of mercury 10 and 50 μg/l (Fig.3). At higher concentration the reducement of cell number was more expressed. During the following days of observation some increase in the number of diatom algae was observed. Pyrophytic algae being in water at the start of experiment gradually decreased being still found till the 10th day while the blue-green algae were not stated any more on the 4th day of experiment.

Experimental results revealed that at both mercury concentrations the development of phytoplankton was inhibited. Some growth in diatom cell number at the end of experiment can be explained by a partial fall in mercury activity due

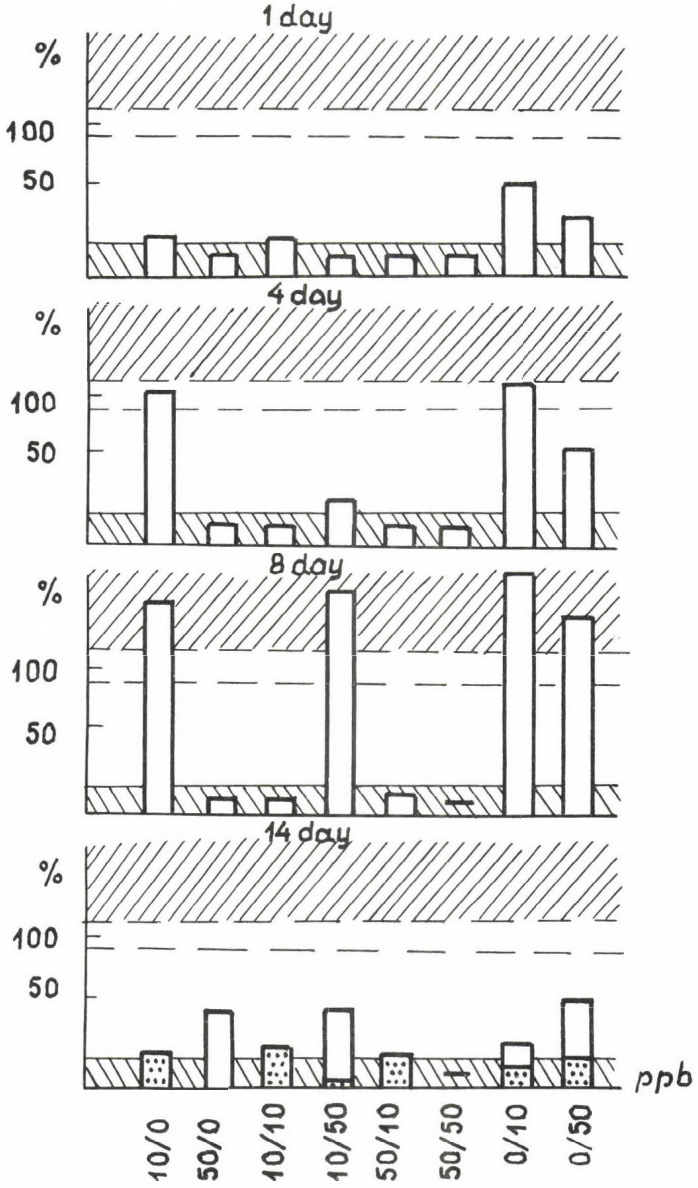


Fig. 2. Effect of mercury and cadmium on the total number of unicellulars.

to the forming of unsoluble compounds and the adsorption of mercury by other water organisms or other factors.

The cadmium concentrations applied by us affected little the development of phytoplankton which was dominated by diatom algae. At 10 μg/l of cadmium a decrease in cell number was observed during the first week of the experiment, and further on their amount remained on the same level. At 50 μg

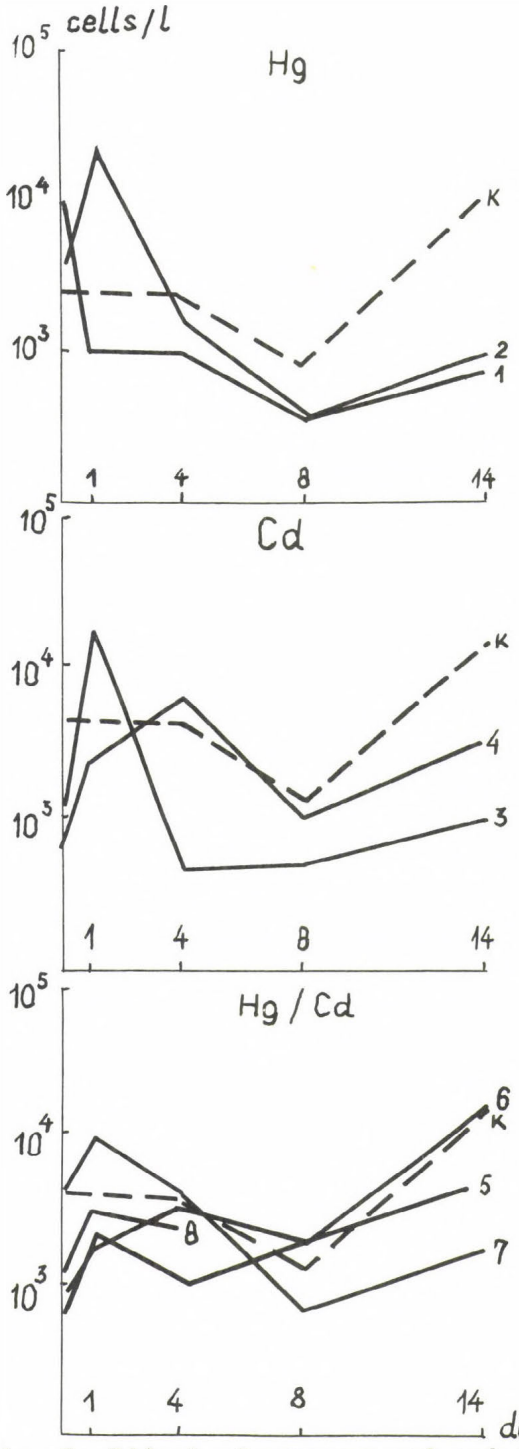


Fig. 3. Effect of mercury and cadmium on the total number of phytoplankton

per 1 of cadmium even a growth in diatom algae was observed during the first 4 days, but the level of the following days remained the same. In between the 4th and the 8th day of the experiment a considerable growth of the blue-green algae was observed, however these algae were not found in the period afterwards. The amount of pyrophytic algae showed no remarkable changes during the whole experimental period. Thus we did not observe any considerable stimulation of phytoplankton development during the experiment, only some inhibition at separate periods in comparison with the control.

The combined effect of mercury and cadmium on phytoplankton was little expressed in comparison with the effect of separate metals, except the inhibition by 50 μg/l of mercury and 10 μg/l of cadmium.

Pigment composition in algae

The results presented in Fig.4 give a rather complicated picture of the mercury and cadmium effect on the pigment composition of algae.

The first four days after mercury was added in concentrations 10 and 50 μg/l a lowered level of chlorophyll "a" was observed in comparison with that of the control.

During the period of the 4th-8th day the amount of chlorophyll "a" decreased in the control and remained constant till the end of the experiment. In experimental bags with the initial concentration of mercury 10 and 50 μg per 1 the level of pigment exceeded that in the control during the investigation period. At cadmium concentrations 10 and 50 μg/l an inhibition of phytoplankton life activity was observed already in the first days of experiment, that was expressed by a "depletion" of algal cells of chlorophyll "a". Inhibiting effect of this metal was felt up to the end of the experiment.

The effect of mercury and cadmium combination was strongly expressed during the first four days. Especially the strong inhibition of metals in 50 and 50 μg/l should be marked during the first days of experiment. About the 8th day of experiment at concentrations 10 and 10, 10 and 50 μg/l a clearly expressed stimulation in the development of algal cells was observed that resulted in the growth of chlorophyll level. A weak stimulation was observed under the effect of mercury and cadmium concentrations 50 and 50, as well as 50 and 10 μg/l. That may be caused by the ability of reparation systems of algal cells to reestablish the level of bioproduction under gradual decrease of metal concentrations in the environment.

Bacterioplankton

The total number of bacteria in experimental bags (Fig.5) with mercury showed almost no changes during the 14 days, while in the experimental bags with cadmium a fall in the total number of bacteria was observed as to the control.

The combined effect of mercury and cadmium had the following response of the bacteria. The development of bacterioplankton at mercury 50 μg/l and cadmium 10 μg/l was inhibited to the end of the experiment. The concentrations

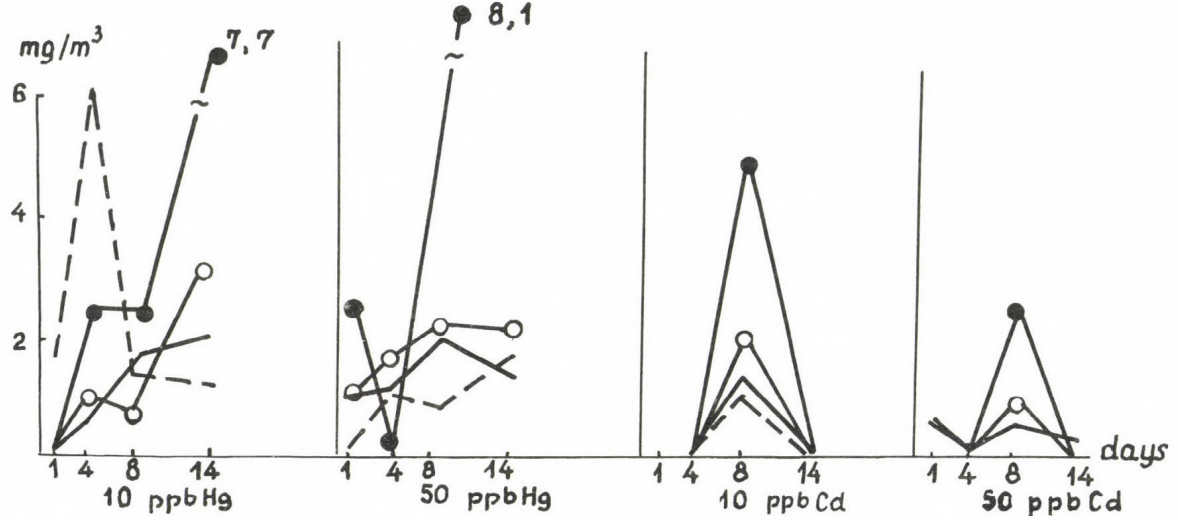


Fig. 4. Effect of mercury and cadmium on phytoplankton pigments

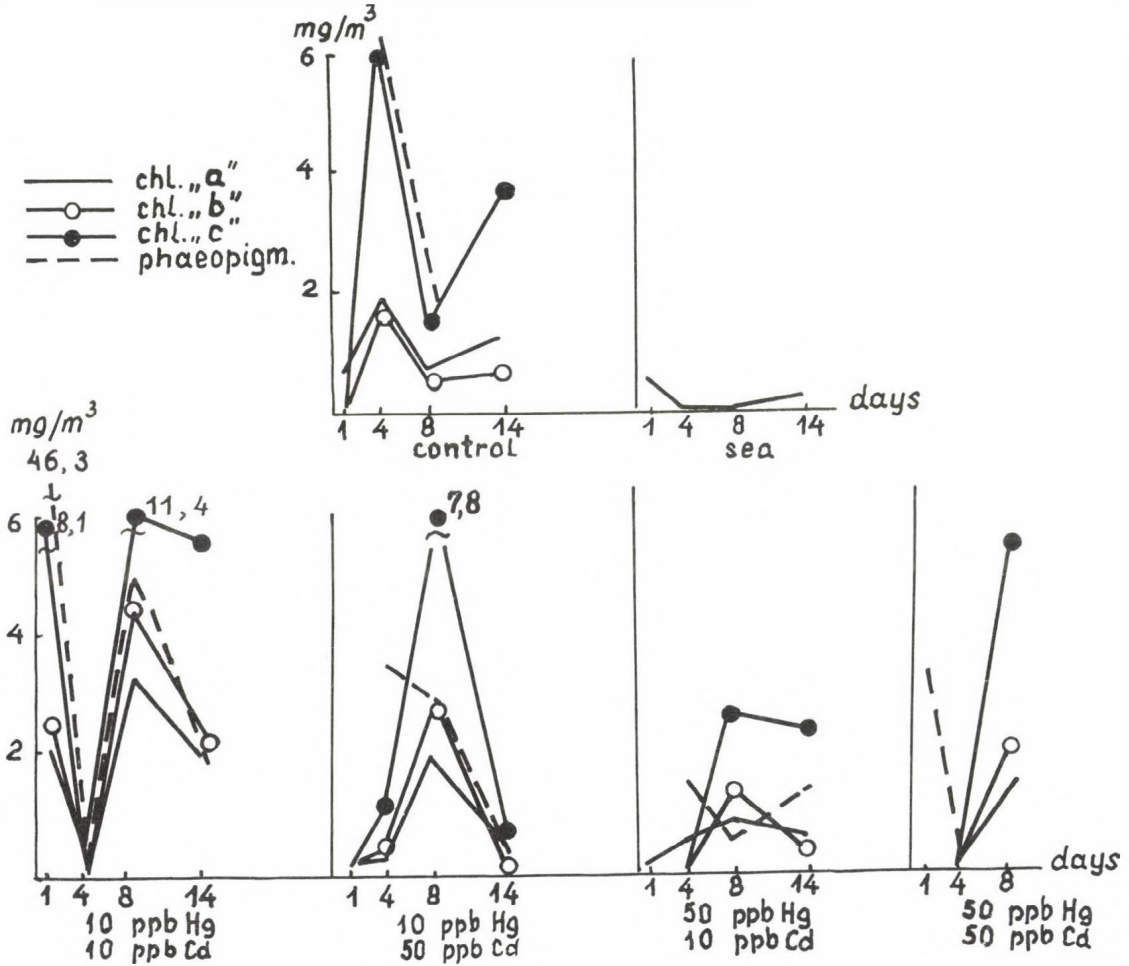
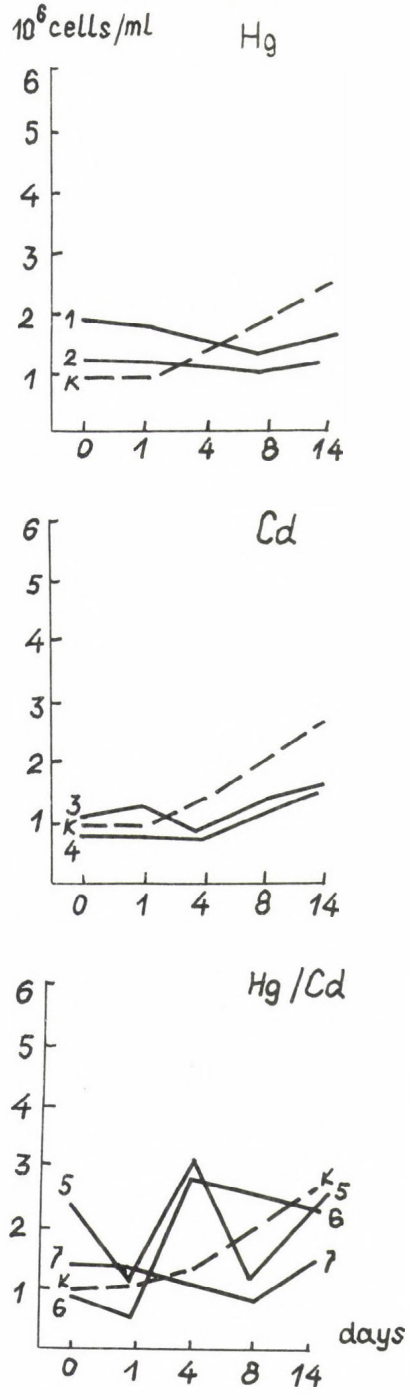


Fig. 4. (continued)



K - control
1 - 10 ppb Hg
2 - 50 ppb Hg

3 - 10 ppb Cd
4 - 50 ppb Cd

5 - 10 ppb Hg
10 ppb Cd
6 - 10 ppb Hg
50 ppb Cd
7 - 50 ppb Hg
10 ppb Cd

Fig. 5. Effect of mercury and cadmium on the total number of bacterioplankton

mercury 10 μg/l and cadmium 10 μg/l, as well as mercury 10 μg per l and cadmium 50 μg/l caused a stimulation of bacterioplankton development.

According to microbiological investigations on changes of the total amount of bacterioplankton and saprophytic bacteria at the presence of various concentrations of one metal and simultaneously of two metals it is difficult to state which concentration and at what period affects most the development of microorganisms. As judged from the experiments, mercury and cadmium more or less cause changes in the development of bacterioplankton.

At the end we should mark that in all the experimental bags the value of pH was lowered. At the beginning of the experiment either in the experimental bags or in the sea pH was 8.9. At the end of the experiment pH of the control bag was 8.4, that of the sea 8.5, but of all the experimental bags with mercury and cadmium concentrations it ranged within 7.8 to 8.3. The lowest pH was found at mercury 50 μg/l (7.9) and at mercury/cadmium - 50/10 μg/l (7.8).

Within 14 days the concentration of mercury and cadmium was falling in all the experimental bags: 50 μg/l concentration of mercury and cadmium showed the greatest changes. Cadmium concentration of 10 μg/l lowered by 10-30%. In spite of the falling in mercury and cadmium concentration plankton development was inhibited in all the experimental bags.

All the selected concentrations of mercury and cadmium separately as well as their combinations inhibited the development of unicellulars, zoo-, phyto- and bacterioplankton either during the whole experiment or periodically. Zooplankton and unicellulars appeared to be the most susceptible to the effect of mercury and cadmium: the first hours after adding of metal in the experimental bags zooplankton and unicellulars showed a sharp response to them. At combined impact of metals the response of zooplankton is less expressed. The combined effect of mercury and cadmium is related not so much with the concentration of a separate metal as of their combination. The species composition of plankton also undergoes changes under the impact of metals.

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DISCUSSION

SHIBER, J: Was there a difference in temperature inside the experimental sacks and outside ocean water? If so, any effects?

SEISUMA, Z: Our experiments were performed in June-August when at our stations temperature of sea water was uniform from the surface to the bottom, as the depth here is only 3-4 m. Temperature of water was the same inside the sacks and in their surroundings, namely at the depth of 1 m.

LORCH, D: Could you please shortly describe the experimental set-up. Were the plastic bags open and were methods employed to ensure mixing?

SEISUMA, Z: The bags were filled with a special funnel through a hole. Having been filled, the bags were closed and suspended at 1 m depth. This method is given in more details in our earlier papers. The bags were swaying in the waves all the time, and so the water was getting mixed. Besides, the bags were turned upside down before taking samples.

V.-BALOGH, K: Cd and Hg increased levels of different forms of chlorophyll and decreased levels of pheopigments. How do you explain this?

SEISUMA, Z: At present we are not able to exactly explain processes of increase and decrease.

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MONITORING OF HEAVY METALS AT INDIVIDUAL LEVEL

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SOME PROBLEMS OF POLLUTION WITH HEAVY METALS UNDER THE ENVIRONMENTAL CONDITIONS OF THE BALTIC SEA

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ABSTRACT

Experimental studies with marine invertebrates from the Western Baltic Sea indicate that accumulation as well as lethal and sublethal effects of heavy metals may be strongly modified by changes of environmental conditions. Modifying influences of temperature and salinity on the effects of cadmium are demonstrated with hydroid polyps and with trochophora larvae of Mytilus edulis. Effects of low oxygen content on the toxicity of copper are reported for a sensitive polychaete and a resistant bivalve species. The last mentioned responds to low copper concentrations in combination with reduced O_2-content with an increased energy demand. - For the purpose of improving biological content monitoring, it is suggested to relate body burdens of pollutants with long biological half lives to the weight of the calcareous shell of bivalves. Results of monitoring of bioavailable cadmium by Cd/shell-wt index of Mytilus edulis from Kiel Fjord exemplify the advantages.

INTRODUCTION

In recent times, among the different types of pollutants, increasing amounts of various heavy metals have been introduced into the marine environment. Following pesticides and related chemicals, heavy metals rank second as potential hazards to marine life. Among these, mercury, cadmium and lead are danger-

ous to aquatic organisms and public health; in addition, copper, zink, silver and chromium have been observed to cause significant damage (Kinne, 1980). The experimental analysis of the effects of pollutants on marine organisms in estuaries, coastal areas and the Baltic Sea must take the prevailing environmental factors into account because they may modify physiological and ecological performances (for literature see Theede, 1980). Among these factors and factor combinations varying temperatures and salinities, oxygen dificiency and nutritional changes may be mentioned. In this report, results of studies from my working group concerning the effects of heavy metals and aspects of biological content monitoring under environmental conditions of the Baltic will be summarized and discussed in context with pertinent literature.

STRESS EFFECTS CAUSED BY CADMIUM AND COPPER IN COMBINATION WITH DIFFERENT ENVIRONMENTAL FACTORS

The assessment of pollution effects may be more difficult on the ecosystem level than on the level of single species. In addition to common criteria on the individual level, such as mortality, reduced growth rates, metabolic activities and reproduction, further indications of stress may be of ecological significance. Stress effects may often be pre steps of changes in competitive performance and of changes in energy flow through the ecosystem.

For the evaluation of such sublethal effects one should keep in mind the difference between the physiological and ecological potential of the organism concerned (see Kinne, 1980). The physiological pontential means the ability of isolated individuals of a species to cope with simulated extreme environmental conditions in the laboratory, whereas the ecological potential comprises the considered capacities of the species in situ. Under optimal biotic and abiotic conditions of laboratory culture (without limitations by competition, e.g. of food and space), the physiological potential may be higher than under

suboptimal in situ conditions. In that case, the effects of pollutants may be small, especially if low concentrations of potentially sublethal doses are applied, to which the organisms may try to adapt during long term exposure and against which detoxifying mechanisms may be activated. If in the reverse case, the sensitivity against different stress factors is higher in the experiment than in situ, the experimental organisms will already live under stress, and then already small doses of additional stressors will cause responses. Different external and internal factors can modify the toxicity of added pollutants, and likewise the sensitivity of an organism against extreme or strongly fluctuating abiotic factors may be affected by pollutants.

Such observations have been made by my working group with hydroid polyps (Scholz et al., 1978; Fischer, 1978; Theede et al., 1979). It is known that thecate polyps are able to respond to extreme environmental stress by unspecific retraction of their hydranths (Kinne 1956, Karbe 1972). When this response was observed in colonies of Laomedea loveni which were exposed for several days to different combinations of temperature, salinity and Cd-concentrations, the polyps reacted very sensitively to Cd-concentrations in the low µg $Cd \cdot l^{-1}$-range. The polyps were most tolerant at low temperature and high salinity combinations (Tab. 1).

In longterm experiments with the athecate Clava multicornis the disappearance of normal feeding behaviour was taken as criterion for injury. The modifying effects of temperature and salinity on the acute toxicity of Cd were similar. But in these cultures, which were supplied with optimal food conditions, higher Cd-levels led to negative responses (Tab. 1). In addition, the modifying effects of toxicity were reduced during the course of a few weeks, and the chronic toxicity reached a level which was attained in short term experiments only at combinations of low salinity and high temperature.

Table 1
Temperature and salinity effects on the acute toxicity of cadmium on two species of hydroid polyps from the Western Baltic Sea. (After Scholz et al.(1978), Fischer (1978), Theede et al. (1979))

Temp.	Sal. °/oo	Upper limit of feeding response *Clava multicornis* days of exposure 7	21	50 % retraction of hydranths *Laomedea loveni* days of exposure 7
		µg $Cd \cdot l^{-1}$		µg $Cd \cdot l^{-1}$
10 °C	10	250	200	11.5
	15	300	200	33.5
	20	375	200	42.5
	25	500	200	57
15 °C	10	200	200	4.3
	15	250	200	9
	20	300	200	15.9
	25	400	200	20.4

The sensitive response of *Laomedea loveni* thus indicates that under extreme environmental conditions, critical changes of behaviour and metabolism may occur at low toxicant levels, whereas under more optimal conditions such levels might be estimated as subcritical or non dangerous. Also in the field, organisms living close to their tolerance limits may suffer from lower additional stress caused by pollution than those under optimal conditions.

It may be assumed that external and internal factors which are able to exert influence on attaining critical effective concentrations of a toxicant within sensitive cells or tissues will modify the toxicity. If we consider cadmium, increased uptake rates at high temperatures and low salinities (found in different estuarine organisms: Phillips, 1976; *Fucus vesiculosus*: Steinhagen-Schneider, 1981; *Zostera marina*: Dieckmann, 1982) may give an explanation for the modifying effects of these factors. According to Mantoura (1978), at low salinities a greater portion of the total amount of dissolved cadmium is available as free bivalent ion, which according to Davies (1978)

and Engel et al. (1981) is supposed to be taken up more easily. However, Simkiss (1983) considers also high lipid solubility of uncharged complexes of the type $(MeCl)^0$ to be of significance.

Studies with larval stages of Mytilus edulis demonstrated stronger negative effects of Cd at lower salinities, too. The optimum salinity for development from trochophora to veliger was shifted to higher salinities at increasing Cd-concentrations. The magnitude of this shift from 25-33°/ooS, when Cd-concentration was increased from 0-50 $\mu g \cdot l^{-1}$, showed that the salinity demand for optimal development may be shifted to such an extent that it is outside the range accessible to Baltic individuals (Lehnberg and Theede, 1979) (Fig. 1).

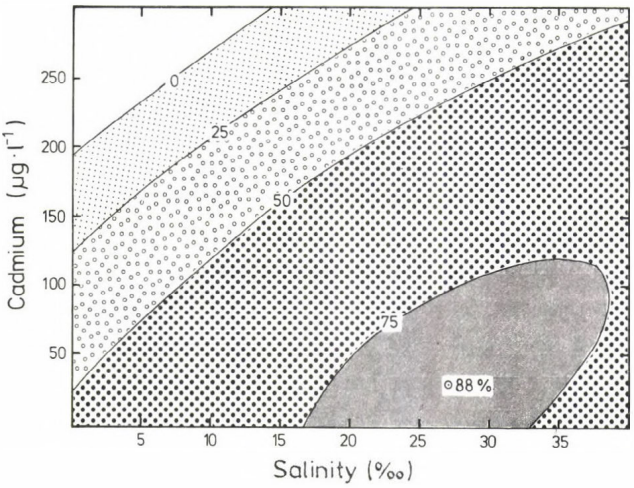

Fig. 1 Percent proportion of Mytilus edulis larvae which had developed from the trochophora to the veliger stage during 6 days´exposure to different Cd-salinity combinations (12° C). (After Lehnberg & Theede, 1979; modified).

A few results of other authors, which point into a similar direction may be mentioned. Gould (1980) studied combined heavy metal-salinity effects and found increased glycolysis in the

lobster Homarus americanus after 30 days of exposure to low salinity and 6 $\mu g \cdot l^{-1}$ Cadmium. Stebbing (1980) found a different sensitivity of the varying reactions of hydroid polyps to sublethal stress. In marine plants, negative effects of relatively low Cd-concentrations have been reported in some cases. In the diatom Coscinodiscus granii, Rabsch and Elbrächter (1980) observed that carbon fixation already started to be negatively affected by Cd concentrations as low as 1.5 $\mu g\ Cd \cdot l^{-1}$. The dinoflagellate Prorocentrum micans responded at about 1.2 $\mu g\ Cd \cdot l^{-1}$ with lowered multiplication rates (Kayser and Sperling, 1980).

In another type of study the oxygen content of the water has been taken into consideration. In the Baltic Sea, periods of oxygen deficiency or even anoxic conditions are common phenomena not only in the depths of the Baltic proper but also in shallower parts (Gerlach, 1983). Moderate hypoxia may be tolerated rather well by several invertebrates (literature: Newell, 1979), but what happens, when it is combined with the additional influence of a pollutant? Considering heavy metals, copper may be present in the Baltic Sea in amounts of up to 8 $\mu g\ Cu\ dm^{-3}$ (Schmid, 1980). The mode of action of copper on aquatic organisms is discussed by Alabaster and Lloyd (1982). When the oxygen content of a stagnant water body is lessened by the respiration of organisms, the dissolved CO_2 content is increased also and, due to this, the balance of the bicarbonate system is shifted towards a lower pH (Broecker, 1974). The decreased pH then leads to changes in the chemical speciation of copper. A greater portion of the dissolved copper will be present in the ionic form (Cu^{2+}) (Zirino and Yamamoto, 1972), which is reported to be more available and more toxic to organisms than the other species of this metal (Pagenkopf et al., 1974; Crecelius et al., 1982; Lewis et al., 1982; Zamuda and Sunda, 1982).

In order to study the effects of low pollutant concentrations in combination with longterm hypoxia, a new device was designed (Neuhoff and Theede, 1983; Neuhoff, 1983). With this equipment

reduced levels of the oxygen in the medium can be kept constant for the purpose of long term experiments. Among the sensitive species to pollution with copper, the polychaete Pectinaria koreni was exposed to different copper concentrations in seawater in combination with different oxygen contents of the medium. Concentrations of 10 µg Cu dm^{-3} applied with low oxygen levels (2.6 cm^3 $O_2 \cdot dm^{-3}$) led to increased mortality within 5 days. In comparison, P. koreni survived much longer at normal oxygen tensions combined with considerable higher Cu-concentrations (70 µg Cu dm^{-3}) (Fig. 2).

Further experiments were carried out with the bivalve Macoma balthica which is abundant in different areas of the Baltic. It is resistant to strong changes of a variety of abiotic factors (Theede, 1983) and may be considered as an euryplastic species. When it was exposed to different Cu-concentrations combined with either 5 or 2.5 cm^3 O_2 dm^{-3}, very low concentrations of copper led to physiological stress effects at low oxygen content.

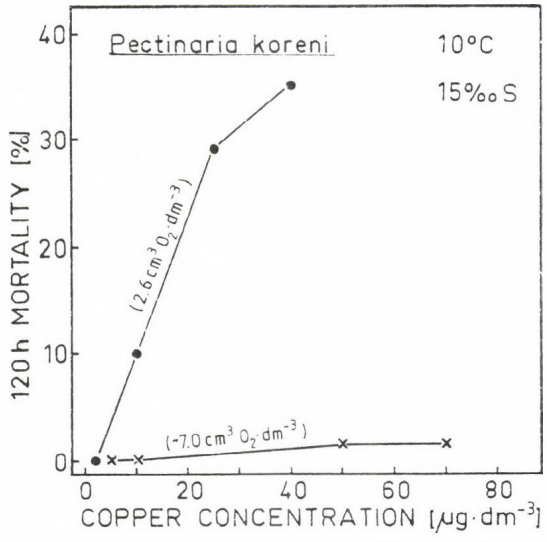

Fig. 2 Mortality of Pectinaria koreni exposed to different Cu-concentrations in combination with reduced and normal oxygen content of the water. (After Neuhoff and Theede, 1983; modified).

Low copper concentrations applied in combination with low oxygen content (and reduced pH of 7.8) in the medium led to relatively higher uptake of the metal (Fig. 3). This may be the result of a better bioavailability of this metal at lower pH-values. Under these conditions the animals showed an increased energy demand, as evidenced by a greater loss of weight and of glycogen, when

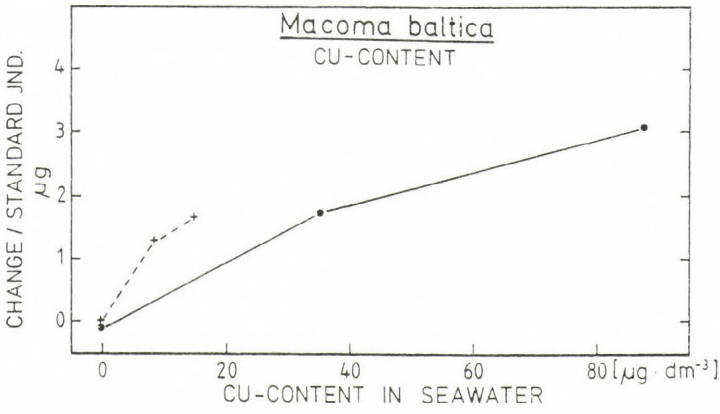

Fig. 3 Macoma balthica. Changes of copper content in the soft body of a "standard individual" with the shell index 1 (length x heigh x thickness)(in cm) after 18 days of exposure to different copper concentrations in water with nearly normal oxygen content (5.0 cm^3 $O_2 \cdot dm^{-3}$) (———) and reduced oxygen content (2.5 cm^3 $O_2 \cdot dm^{-3}$) (- - -); (10° C; 15 °/oo S). (After data of Neuhoff, 1983).

kept for 18 days under such conditions without food (Fig. 4). By comparison, in an uncontaminated medium at normal oxygen condition, almost no weight loss was found. At normal oxygen tension comparable glycogen and weight losses occur only at considerably higher concentrations of copper.

In addition, the influence of copper on the adenylate energy charge (AEC) was measured at different oxygen concentrations. The AEC has been used successfully by different authors to characterize the energetic state of cells and organisms in relation to

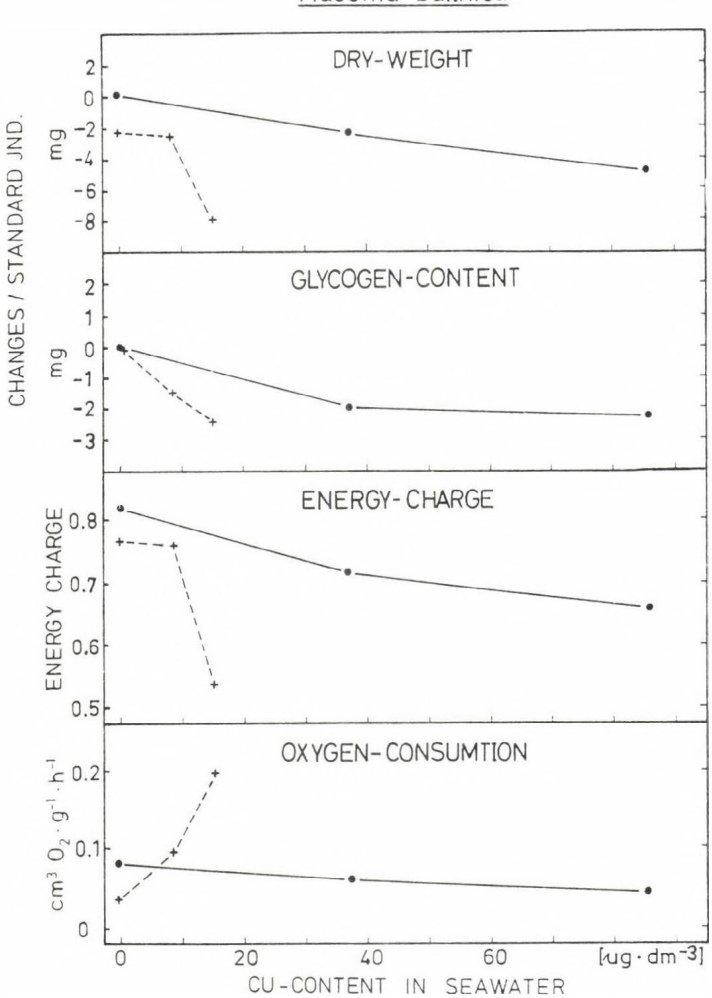

Fig. 4 Macoma balthica. Changes of dry weight and glycogen content in the soft body of a "standard individual" during the experiment, and values for adenylate energy charge and oxygen consumption after 18 days of exposure to different copper concentrations and O_2 contents in sea water.
2.5 cm^3 $O_2 \cdot dm^{-3}$: - - -; 5.0 cm^3 $O_2 \cdot dm^{-3}$ ——
(10° C; 15°/oo S). (After data of Neuhoff, 1983).

environmental conditions (for literature cons. Neuhoff, 1983). It describes the amount of metabolic energy potentially available. Values between 0.8 and 1 are assumed to indicate optimal environmental conditions, those below 0.8 suboptimal conditions, and values below 0.5 should indicate lethal conditions.

Adenylate energy charge values in Macoma balthica indicated optimal conditions only in uncontaminated media and in combination with a sufficient oxygen supply (Fig. 4). Increasing copper concentrations up to 15 µg Cu dm^{-3}, combined with hypoxia, resulted in a marked deterioration of metabolism, almost down to the level of irreversible damage, whereas even much higher Cu-concentrations at a normal oxygen level merely led to a decrease of AEC towards "stress indicating values". In addition, greatly increased oxygen consumption is observed in longterm measurements with low copper-low oxygen conditions. This contrasts with a slow reduction of oxygen consumption at normal oxygen conditions even if copper concentrations are increased to 86 µg Cu·dm^{-3}.

Altogether, the higher energy demand (concluded from weight- and glycogen-loss), the strong reduction of AEC and the increase of oxygen consumption indicate stress effects of a combination of low copper concentrations (8-15 µg Cu dm^{-3}) and hypoxia.

ACCUMULATION AND BIOLOGICAL MONITORING OF CADMIUM

For the purpose of biological content monitoring of the bioavailable fraction of heavy metals and other pollutants in the sea marine mollusks have been used by different authors (for literature cons. Phillips, 1977; 1980; Fischer, 1983). In coastal areas of the temperature zone, one suitable species is the blue mussel Mytilus edulis because of its abundance and its high capacity to concentrate many elements to a considerable degree without any noticeable signs of negative physiological effects (Scholz, 1980; Köhler and Riisgaard, 1982). Poulsen et

al. (1982) found that body loads of cadmium up to 150 ppm caused no effects on either clearance, ingestion, assimilation, respiration and growth of the mussel within two weeks.

However, the indicator ability of mussels is restricted because local and seasonal differences in body weight may lead to great variability of heavy metal concentrations (e.g. Phillips, 1976a). The fact that the total content of heavy metals in the soft body varies considerably less than the tissue concentration (Zaroogian, 1980), offered a good possibility to try improvements to biological monitoring. Several authors tried different approaches in this respect (see Fischer, 1984a). Fischer (1983) demonstrated that the variability of cadmium concentration due to the variations in soft body weight could be eliminated by relating cadmium body burden to shell weight. This author defined a Cd/shell-weight index which is derived from the logged regression line of the equation "$Cd = a \cdot Shell\text{-}wt^b$". This index indicates the cadmium content (µg) in the soft body of a mussel which has a shell weight of 1 g.

Field experiments have shown that within the same population this index is independent of variables which modify the condition of the mussel, as individual size, tidal exposure, spawning and nutrient supply. Growth experiments proved in addition that temperature and oxygen saturation do not change this index. There was also no detectable influence of concomitant 30-50 fold elevated Zn-concentrations on Cd-accumulation. However, reduced salinity below $15^{o}/_{oo}S$ led to stronger retardation of shell growth than of the soft body. In that case Cd/shell-wt index is higher. By use of it, Fischer (1983, 1984a, b) improved biological monitoring of cadmium in Kiel Fjord. Whereas nine years ago, conventional concentration analysis could be used to detect the decline of Cd-pollution from the innermost part of Kiel habour ($\sim$ 30µg $Cd \cdot g^{-1}$ tissue dw) to Kiel Bight ($\sim$ 2 µg $Cd \cdot g^{-1}$ tissue dw)(Theede et al., 1979a), decreased levels of Cd-pollution in 1980 and the following years showed the necessity of improving

the sensitivity of the method for biological content monitoring. Even at low scale pollution, the mentioned Cd/shell-wt index proved to be still a good indicator for bioavailable environmental cadmium. It indicated moderate pollution in Kiel harbour and near Kiel sewage discharge. A decrease at certain localities down to levels below the baseline of Kiel Bight was interpreted to reflect self-purification processes of the euthrophicated areas. In addition seasonal anoxic conditions and formation of H_2S may lead to temporary reduction of the amount of bioavailable Cd in the water column (Fischer, 1984b). This was also reflected by the Cd/shell-wt index.

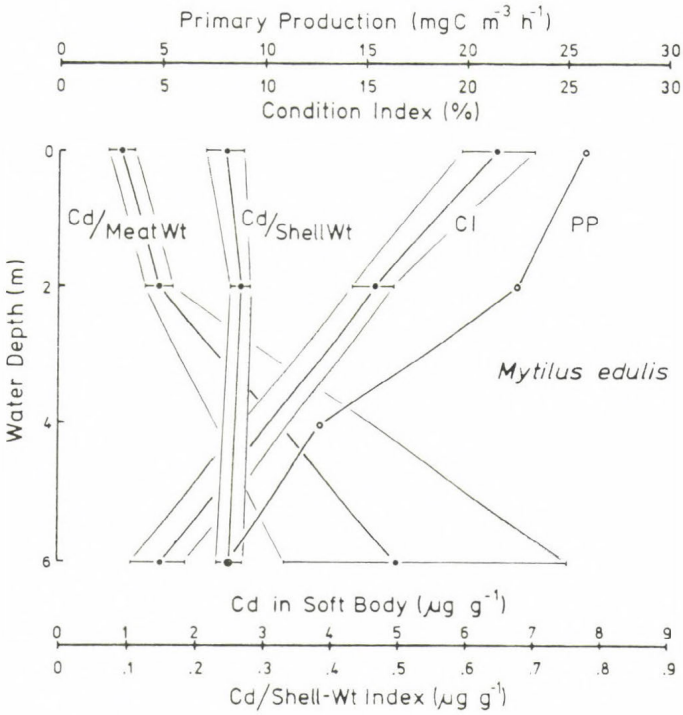

Fig. 5 Cd/shell-wt index of Mytilus edulis from Kiel Fjord reflects uniform Cd availability with depth, whereas depth dependent differences of Cd concentrations are the result of changes of condition index. (After Fischer, 1983).

It is interesting to note that the relation of Cd-body burden and shell-weight in given mussel populations was steady over the whole size range. This points to accumulation during whole life. Good examples to demonstrate the effect of growth and condition index of mussels on their Cd-concentration are studies at different depths of one locality (Fig. 5). In the mentioned case, increase of Cd-concentration with depth reflects decrease of condition index, which is a result of lower nutrient supply. However, the Cd/shell-wt ratio indicates uniform Cd-availability with depth. This result points to stronger uptake of Cd from water than from food.

Fischer (1983, 1984a) suggests to relate the amounts of other pollutants with long biological half lives in the soft bodies of mussels with the weight of the calcareous shell for the purpose of a more exact biological monitoring in future.

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DISCUSSION

BRIX, H: Have you any experience on using this shell-ratio index with other metals than cadmium?

THEEDE, H: The heavy metal-shell weight index should be applicable for metals with long biological half lives. This is stated by preliminary studies with lead, whereas the situation with copper seems to be more complicated.

SHIBER, J.G: Have you made any measurements of cadmium in the presence of one or more other metals (added to the experimental medium)?

THEEDE, H: Such studies have been done with addition of Zn. When Zn was added 30 to 50 times that of the Cd-levels, no clear influence was detectable. Only at higher Zn-concentrations was the Cd-uptake negatively affected.

SALÁNKI, J: Your remark that there was no physiological change in the mussel's filtering and growth in Cd-rich water seems to be questionable. Some years ago I conducted experiments on Mytilus in the Mediterranian and observed very expressed changes in the filtration activity, when rest periods were prolonged and activity was reduced (Comp.Biochem.Physiol. 18, 829-843).

THEEDE, H: The cited results of a recent paper by Poulsen et al. (1982) demonstrate that the mussel is able to accumulate high amounts of cadmium without signs of damage within a short time. The experimentally used Cd-concentrations were much higher than occur in natural marine environments. However, I agree with you that you may already obtain negative effects with even lower Cd-concentrations in the medium, if the time of exposure will be long enough.

WACHS, B: An essential prerequisite for monitoring is the knowledge about the behaviour by differentiation of the important ecofactors. Here we have very clearly seen the influence of those components as pH, temperature and O_2 content, which are, in general, only possible to differentiate in the lab.With such experiences we can start field investigations or measurements in the natural ecosystems.

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GONADOTOXIC AND EMBRYOTOXIC EFFECTS OF CADMIUM IN SEA URCHINS

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The present communication reviews the studies performed by our research group during the recent 5 years on the influence of cadmium upon reproduction in sea urchins.

In 1937, Waterman established a scale of toxicity for various metal salts in terms of their influence upon sea urchin embryos. In 60-70s, sea urchins were adopted as a suitable object for studying the influence of various environmental factors upon early developmental stages and were first used in toxicological and pharmacological testing (Okubo,Okubo,1962;Kobayashi, 1971,1977;Buznikov,Podmarev, 1975; and others).

Availability of a large number of gametes and synchronously developing embryos, simplicity of their incubation under controlled conditions,morphological and chronological clarity in changing of developmental stages, and high sensitivity of embryos to increased contents of metals in medium - all these advantages give a unique opportunity to use sea urchin eggs as an indicator in estimating sea pollution.

Using a routine approach to estimation of toxic effect upon early ontogenetic stages in sea urchins, we studied

survival and viability of Strongylocentrotus intermedius embryos after artificial insemination of egg cells in sea water with cadmium concentrations of 5 µg/l to 5 mg/l (Table 1).

Table 1

Occurrence of anomalies in embryonal development of sea urchins under cadmium stress, in %

Cadmium concentration, mg/l	Developmental stages				
	Fertilization membrane	Two blastomeres	Blastula	Gastrula	Pluteus
Control	1.3	1.6	1.8	1.2	1.7
5	5.5	8.5	9.8	66.4	100
2.5	9.1	8.3	8.8	36.0	100
0.5	0.6	7.1	7.5	28.9	32.1
0.1	9.2	13.0	12.0	16.0	16.3
0.04	7.1	6.7	8.5	8.2	5.2
0.005	1.7	0.7	1.7	1.7	1.2

It has been previously reported (Vlasova, Khristoforova, 1982) that, irrespective of cadmium load before hatching, which occurs in the late blastula, embryos remain little damaged by cadmium stress. In our experiment, maximal number of anomalies and highest mortality were registered in the moment of transformation of embryos to larvae. At high cadmium concentrations in the medium (5 and 2.5 mg/l), we observed mass extinction of plutei. However, at the next tested concentration of 0.5 mg/l, two thirds of embryos developed to normal plutei. The concentration of 5 µg/l did

not seem to affect the development of fertilized egg cells. With a new parent pair used in each series of the experiment, we have found already in preliminary study, that embryogenesis of sea urchins under cadmium stress depends both on concentration of toxic matter and on the initial state of sex products. A low quality of egg cells changed the developmental pattern wholly, in this case a great number of anomalous embryos and their mortality were observed in different degree through all the stages of embryogenesis (Fig.1).

The surge for understanding the processes affecting quality of sex products has led us from short-term observations of early ontogenetic stages to long-term experiments on gametogenesis in mature individuals. Sea urchins are also a suitable object for studying the growth of sex cells, since the entire development of sea urchin gametes from ovogonia to ripe egg cells occurs within the gonad (Gnezdilova et al. 1979). This allows a researcher to follow a harmful effect of toxicant through various stages of differentiating sex cells (Fig.2).

In our long-term experiments with sexually mature individuals maintained in cadmium solutions, we observed the behaviour of sea urchins, studied the dynamics of accumulation of cadmium in tissues and histological changes in gonads, and estimated the effect of preliminary chronic effect of this toxicant upon mature animals by the quality of their offspring, developing under normal conditions (Khristoforova et al., 1984).

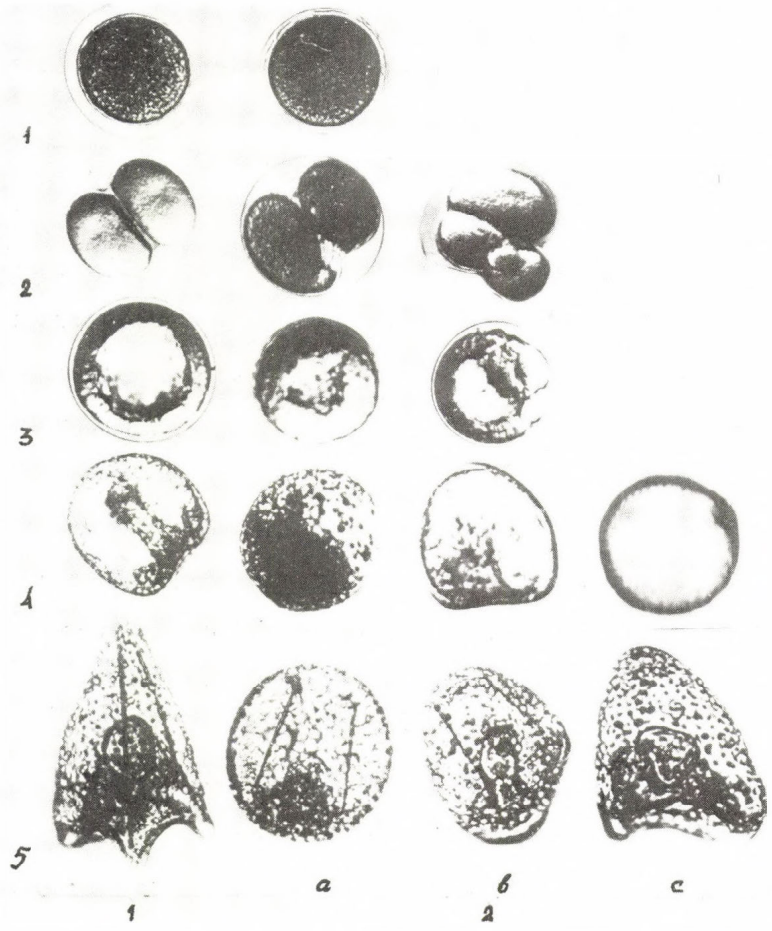

Fig.1 Stages of embryogenesis in the sea urchin under normal conditions and under cadmium stress

A - normal embryogenesis: 1 - formation of fertilization membrane, 2 - division to 2 blastomeres,3 - blastula, 4 - gastrula,5 - pluteus. B - anomalous embryogenesis: 1 - fertilization membrane of irregular form, 2 - division to 2 unequal and 3 blastomeres, 3 - anomalous blastulae,4 - anomalous gastrulae:endogastrula(a), gastrula with broad and short archenteron (b),gastrula without archenteron(c); 5 - anomalous

plutei: round(a) or irregular in form, with spreading spicules or without spicules (b,c).

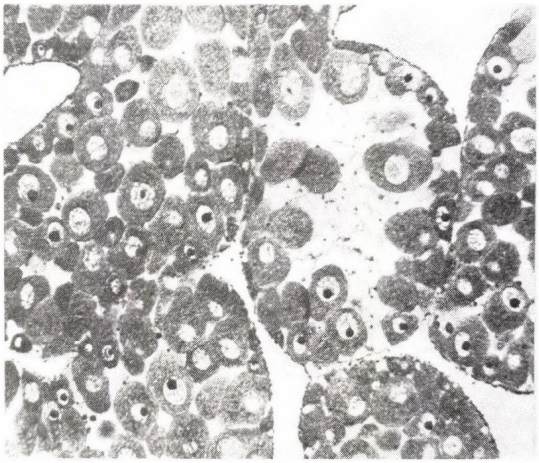

Fig.2 A normal sea urchin gonad. Stain: hematoxylin Caracci. Magnification 120 X

The greatest amount of cadmium was accumulated in the intestine of the test animals. In females, after 10 days of exposure to 0.1, 0.5 and 1.0 mg Cd/l concentrations, cadmium contents were found to be 16.0, 281 and 323 µg/g dry matter, respectively, while control animals contained only 3 µg Cd/g. During three decades, cadmium concentration in the intestine of test animals increased 1.5 - 3 times. The intestine accumulated greater Cd amounts, than gonads did, and male individuals accumulated more than females (Table 2).

Histological investigations of the state of the gonads of sea urchins exposed to high concentrations of cadmium ($\geqslant$1 mg/l) showed that many growing ovocytes and ripe egg cells underwent resorption (Fig.3).

Degenerative changes in the ovaries of test animals were seen in an increase of the number of gigantic globules in ovarian acini, serving as heterophagosomes at resorption of sex cells. In nature, an increase in the number of acces-

Table 2

Dynamics of accumulation of cadmium in sea urchin gonads

(µg/g dry matter)

De-	Cadmium concentrations, mg/l							
ca-	Control		0.1		0.5		1	
des								
1	n.d.	n.d.	n.d.	n.d.	12.53	5.80	15.09	36.6
2	n.d.	0.25	0.75	1.09	8.07	15.65	14.09	57.5
3	n.d.	0.20	2.97	1.01	10.08	40.11		
4	n.d.		3.32	3.81				

n.d.=not detected

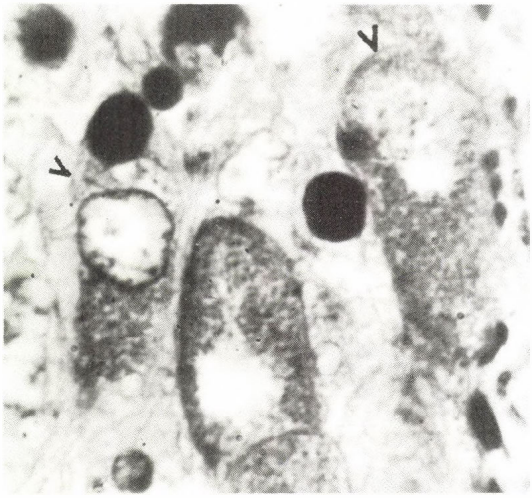

Fig.3 Resorption of ovocytes in test animals.
Stain: hematoxylin Caracci. Magnification 240 x

sory cells including the gigantic globules occurs in autumn and winter, when gonad resorption processes predominate.

Gamete resorption was accompanied by obviously poor fertilization of egg cells obtained from test animals at the end of the first decade of exposure, and this suggests a low quality of sex products in test animals (Table 3).

Table 3

Embryogenesis after exposure of mature sea urchins to different cadmium concentrations

Cadmium concentration, mg/l	Normal embryos (%) for developmental stages				
	Fertilization membrane	Two blastomeres	Blastula	Gastrula	Pluteus
Control	96.4	96.4	94.2	93.3	94.5
	97.3	97.3	94.0	99.5	97.8
0.1	48.0	88.0	64.8	84.4	0.0
	96.2	88.9	77.2	79.5	0.0
	91.0	90.5	76.5	87.1	0.0
0.5	51.1	71.0	81.2	57.4	0.0
	86.7	90.5	80.5	74.4	0.0
1.0	36.0	91.0	67.8	55.7	0.0

However, at Cd concentration of 0.5 mg/l, the animals were capable to enhance generative processes in gonads, which led to an increase in the number of small ovocytes and to enlargement of fertilized egg cells. An enhancement of generative processes in gonads resulted also from a long-term exposure of sea urchins to 0.1 mg Cd/l. However, in spite of an apparent well-being of sea urchins in aquarium with sea

water of this cadmium concentration, insignificant accumulation of Cd in gonads, and an absence of appreciable histological changes in the development of ovocytes, embryogenesis resulted in anomalous larvae. All this suggests, that at a long-term exposure of S.intermedius to a sublethal Cd concentration of 0.1 mg/l, some histopatological changes occurred in ovaries of test animals, which were not detectable with a light microscope, but resulted in formation of anomalous offspring. It was obvious, that investigations at a higher level were needed, with application of electron microscopy.

Electron microscopic examination of ovocytes in test animals showed the appearance of vacuoles and spaces in their ergastoplasm, which were not found in the control. Mitochondrial membranes vanished, nucleus lost its regular form. Chromatin condensed near the nuclear envelope (Fig.4A).Some alterations could be also observed in accessory cells: their cytoplasm disintegrated, included vacuoles and voluminous hollows, cell borders vanished. Chromatin condensed in the nucleus, the latter became pycnotized (Fig. 4B).

At longer exposure of animals to cadmium concentrations, morphological changes in cells became more apparent. In gonads of control animals, resorption of ovocytes and destruction of accessory cells were not observed.

We used the histochemical reaction of sulphide-silver to detect cadmium in sea urchin ovaries. At maintenance of animals in cadmium-contaminated water, a deposit of tiny black granules was distinctly seen in sex glands. Rare sing-

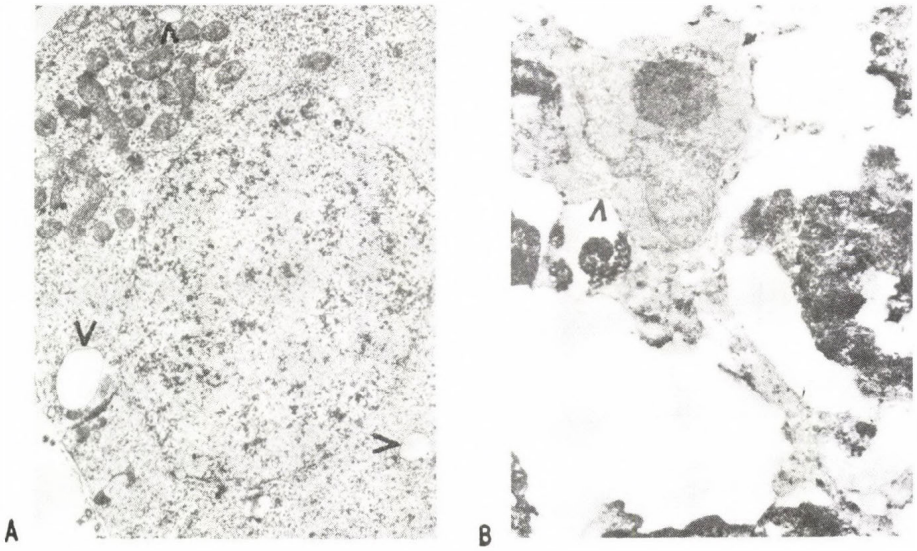

Fig. 4 Resorption of ovocytes in accessory cells. Electron micrographs, 23 000 X

A - Vacuoles in ovocytes of early protoplasmatic growth. B - Destruction of accessory cells.

le granules without any regular distribution pattern were observed in the cytoplasm of sex and accessory cells in gonads of control animals, while in the test group the deposit was more voluminous and showed a characteristic pattern. In the beginning of exposure, small granules occurred mostly in gonad wall and in intercellular spaces (Fig. 5 A,B).

In accessory cells, the deposit condensed selectively in globules (Fig. 6A). Later on, cadmium appeared in sex cells of various growth and maturation stages, it was presumably detected in young cells of early protoplasmatic growth (Fig. 6B). The deposit was less voluminous in ovocytes of later growth stages and in ripe egg cells. In nuclei of sex

cells,localization of the deposit was most distinct.

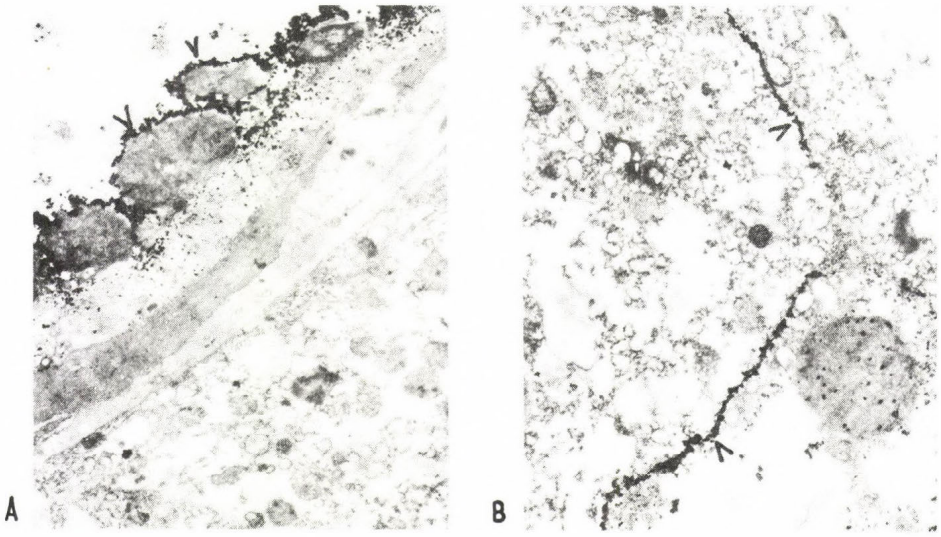

Fig. 5 Localization of the deposit in sea urchin gonads. Sulphide-silver reaction.Electron micrograph, 15 000 X . A - Distribution of the deposit over an external gonad wall. B - Accumulation of cadmium in intercellular spaces.

At less intensive cadmium stress (e.g. 80 µg/l), the deposit was found after 40-day-long exposure mostly in globules of accessory cells. The deposit was not found either in gonad walls or in intercellular spaces and on cellular membrane surface.

Thus, our study of accumulation, distribution and transformation of cadmium in sex glands of sea urchins showed the pathway of this toxicant within the gonad, which results in irreversible disturbances of gametogenesis : gonad wall - accessory cells - ovocytes - ovocyte nuclei.

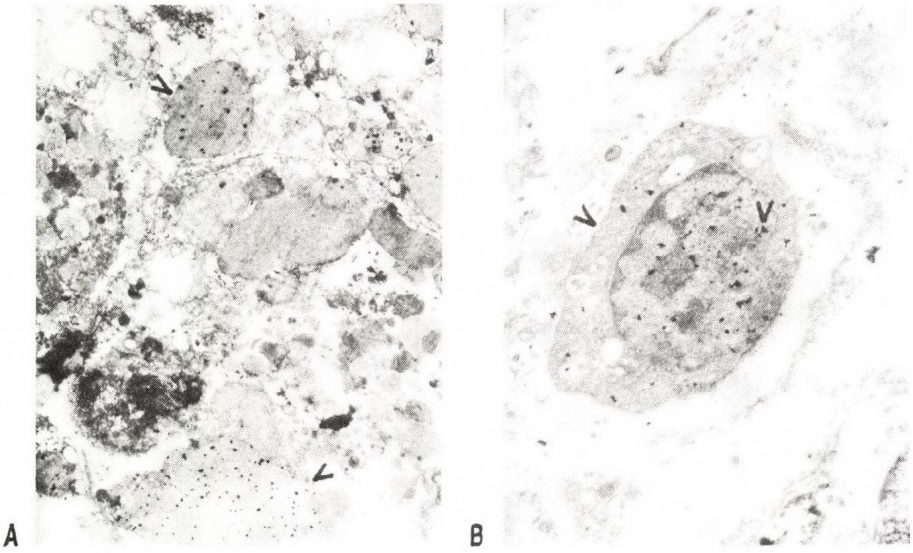

Fig.6 Appearance of the deposit in the cells of sea urchin sea gland. A - Distribution in globules of accessory cells. B - Localization in ovocytes of early protoplasmic growth. Electron micrograph, 20 000 X

An apparently significant concentration of cadmium within accessory cells suggest that the accessory cells have a barrier function. Only at disturbances of this barrier, cadmium intrudes a proper sex cell.

Summarizing, we would like to emphasize once again that in spite of survival of mature sea urchins in cadmium solution of sublethal concentrations and insignificant accumulation of cadmium in gonads, appreciable intercellular transformations occur in gonad cells, which result in development of anomalous offspring.

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DISCUSSION

SHIBER, J: Please explain the possible reason/s/ why the gastrulae are most affected by all the studied Cd concentrations.

GNEZDILOVA, S: During the onthogenesis of the sea urchin embryos the gastrulae are the first formswhich get into direct contact with the sea water. Therefore the dissolved cadmium causes more significant morphological changes at this stage of the embryogenesis, than at earlier ones.

WEIS, P: The stimulation of oögenesis which you observed at a low concentration of Cd is similar to what has been observed in other growing systems by other investigators; using a sub-toxic level of Cu, Sanders et al /1983/ found increased growth of crab larvae and, with Cd, we have enhanced the rate of fin regeneration in killifish. This stimulation of growth by a low level of a toxic material is termed "hormesis", and has been most extensively studied by Anthony Stebbing in England. It is not well understood.

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THE INFLUENCE OF SIZE UPON THE CONCENTRATIONS OF Cd, Cr, Cu, Hg, Pb and Zn IN THE COMMON MUSSEL (*MYTILUS EDULIS* L.)

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Risskov, Denmark

ABSTRACT

The effect of mussel size upon the concentrations of cadmium, chromium, copper, mercury, lead and zinc in the soft tissues and shells of the common mussel (Mytilus edulis L.) was investigated. The mean concentrations of the metals in the mussels decreased in the order: Zn > Pb > Cu > Cr > Cd > Hg. On a dry weight basis the concentration levels of all metals were significantly greater in the soft tissues than in the shells. The results of the present investigation suggest, that shells of Mytilus edulis are of no practical use in the monitoring of the metals investigated.

Three different kinds of relationships between metal concentration and mussel size (soft tissue weight or shell weight) were recorded:

- The concentration of Cr in the soft tissue and those of Cd, Cr, Hg and Zn in the shells decreased significantly with size.
- The concentrations of Hg and Pb in the soft tissues increased significantly with size.
- The concentrations of Cd, Cu and Zn in the soft tissues and those of Cu and Pb in the shells were independent of mussel size.

The observed relationships are discussed in relation to physiological and environmental factors, and the implication of the results for the use of the common mussel for monitoring heavy metal pollution are discussed.

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INTRODUCTION

The common mussel (Mytilus edulis L.) is widely used for monitoring heavy metal pollution in coastal areas (e.g. Phillips, 1977a; Davies & Pirie, 1980; Popham et al., 1980; Lyngby & Brix, 1983), and it has been proposed, that this organism should be used in a global monitoring program: "The Mussel Watch" (Goldberg, 1975; Goldberg et al., 1978).

A prerequisite of using Mytilus for monitoring heavy metal pollution is that the heavy metal concentrations in the mussels reflect the concentration levels in the environment, i.e. the heavy metal pollution. There are, however, other factors, such as body size, reproductive stage, salinity, temperature, position in the water column, etc., which have been shown to have an influence on the heavy metal concentrations in the mussels (e.g. Phillips, 1976; 1977b; 1980; Davenport, 1977; George & Coombs, 1977; George et al., 1978). Therefore, in order to be able to take such environmental and physiological factors into account in the evaluation of data from a monitoring study, it is important to know how the different factors influence the metal concentrations in the mussels.

Some authors have suggested, that bivalve shells are superior to soft tissues for monitoring heavy metal pollution (Ferrell et al., 1973; Koide et al., 1982). However, there is a lack of documentation concerning this topic.

The purpose of the present investigation was to study the influence of mussel size upon the concentrations of cadmium, chromium, copper, mercury, lead and zinc in soft tissues and shells of the common mussel (Mytilus edulis L.) in order to elucidate the significance of size-dependent variations in the application of Mytilus as an indicator organism.

MATERIALS AND METHODS

Sampling and analytical procedure:

The mussels used in this investigation were collected on October 13th, 1982, in the Limfjord, Denmark, near the city of Aalborg. The concentration levels of copper, mercury and lead in the sediment, and the concentration levels of mercury and lead in the soft tissues of mussels are known to be enhanced at the sampling location compared to the background levels in the Limfjord (Tab. 1).

Approximately 150 mussels of different size were sampled, and any barnacles and algae on the shells were removed. The mussels were kept in running seawater for 24 hours after collection to clear their guts, and thereafter frozen.

In the laboratory, 25 mussels covering a wide size-range (shell length: 26-78 mm) were chosen for analysis of mercury, and 25 (shell length: 24-81 mm) for analysis of Cd, Cr, Cu, Pb and Zn.

Table 1. Mean concentrations of Cd, Cr, Cu, Hg, Pb and Zn in the upper 5-cm of the sediment (µg/g Loss on Ignition) and in the soft tissues of Mytilus edulis L. (µg/g dry weight) at the sampling location compared to the background levels in the Limfjord (Brix & Lyngby, 1984).

	Sediment		Mytilus	
	sampling location (µg/g LI)	background level (µg/g LI)	sampling location (µg/g dw)	background level (µg/g dw)
Cd	6.4	6.2	0.97	1.00
Cr	188	187	2.3	1.6
Cu	377	153	11.7	9.6
Hg	3.5	0.6	0.31	0.11
Pb	950	161	18.6	1.4
Zn	1583	1013	216	120

Upon thawing, the mussels were placed on their edges so that the intrashell water could drain. Thereafter the soft tissues were dissected from the shells, weighed, and digested in either HNO_3/H_2SO_4 with $K_2Cr_2O_7$ added (for analysis of mercury) or HNO_3/H_2O_2 (for analysis of Cd, Cr, Cu, Pb and Zn).

The shells were rinsed in demineralized water, weighed, and the shell length noted. One of the shells were digested in HNO_3/H_2O_2.

Mercury was analyzed by the cold vapor technique, and Cd, Cr, Cu and Pb by flameless AAS (Perkin-Elmer 305B, HGA-74, Deuterium background corrector). Zinc was analyzed by flame-AAS in an air-acetylene flame (Perkin-Elmer 503). The method of standard addition was used for all determinations.

The metal concentrations in the soft tissues are in this work consistently given on basis of wet weights. Statistical computations were made on log-transformed data.

Data treatment:

Boyden (1977) has given a minute matematical description of different kinds of relationships between metal concentrations/contents in mussels and size of the mussels. These relationships are briefly summarized below.

Metal concentrations in mussels may either increase with mussel size, decrease with mussel size, or be independent of mussel size. Often the observed relationships between metal concentrations/contents and mussel size are of a curved nature.

The relationship between metal content in the mussels and size may be described in the following way. Total metal content per individual (Y) is related to mussel weight (X) as a power function:

$$(1) \qquad Y = aX^b$$

which upon logarithmic transformation yields the equation:

$$(2) \qquad \log Y = \log(a) + b\log(X)$$

This expression (2) describes a straight line with the slope b and the intercept log(a). Three different kinds of linear relationships can be derived from the magnitude of the slope b:

(a) $b < 1$: The linear relation is curved with larger individuals containing less metal than would be expected if metal content was directly related to mussel size.

(b) $b = 1$: The linear relation is a straight line, i.e. the metal content in the mussels is directly related to the mussel size.

(c) $b > 1$: The linear relation is curved with larger individuals containing more metal than would be expected if metal content was directly related to mussel size.

Similarly the metal concentration (Y') is related to mussel weight (X) as:

$$(3) \qquad Y' = \frac{Y}{X} = \frac{aX^b}{X} = aX^{(b-1)}$$

which upon logarithmic transformation yields the equation:

$$(4) \qquad \log(Y') = \log(a) + (b-1)\log(X)$$

The slope $(b-1)=b'$ of this regression line can, as was the case for the slope b in equation (2), be interpreted in a similar way:

(a) $b' < 0$: The metal concentration decrease with mussel size.

(b) $b' = 0$: The metal concentration is independent of mussel size.

(c) $b' > 0$: The metal concentration increase with mussel size.

The use of equation (2) and (4) therefore gives equivalent interpretations of the data.

It has been shown (Boyden, 1977), that the linear relationships between metal content or concentration and mussel size often are curved. However, logarithmic plots usually give straight lines. Therefore only logarithmic relationships are considered in the present paper. Correlations of metal concentrations with shell length were in this study generally similar to correlations with weight. Only weight relationships are presented.

RESULTS

1. Mean metal concentrations

In Table 2 are the geometric mean concentrations of the six metals in soft tissues and shells of the mussels listed. The concentration of the metals in both soft tissues and shells decreased in the order: Zn > Pb > Cu > Cr > Cd > Hg. On a wet weight basis the mean concentrations of Hg, Cd and Zn in the soft tissues were respectively 10, 5.3 and 4.4 times greater than the mean concentrations in the shells, whereas the concentrations of Cr, Cu and Pb in the soft tissues were of the same magnitude as the concentrations in the shells. On a dry weight basis, however, the figures for the concentrations in the soft tissues would be 5-6 times greater than on basis of wet weights, while the levels in the shells would be approximately the same. This means, that on basis of dry weights the concentrations of all metals investigated were 4-50 times greater in the soft tissues than in the shells.

Comparing the mean metal concentrations in the soft tissues (dry weight: 15-20% of wet weight) with the background levels in the Limfjord (Table 1), it is seen, that the mercury and lead concentrations in the soft tissues were respectively 3 and 15 times higher than the background levels in the Limfjord. The concentration levels of Cd, Cr, Cu, and Zn did not deviate significantly from the background levels in the Limfjord.

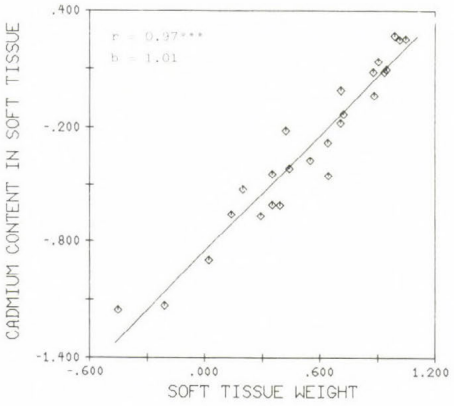
The coefficients of variation were generally great, especially for Pb and Zn in the soft tissues and Cd and Zn in the shells (Table 2). Copper is the metal showing least variation.

Table 2: Geometric mean concentrations ($\bar{x}_{geo}$) and asymmetric coefficients of variation (C.V. %) of the six metals in soft tissues and shells of *Mytilus edulis* L. (n=25).

	Soft tissues		Shells		
	$\bar{x}_{geo}$ (µg/g ww)	C.V. (%)	$\bar{x}_{geo}$ (µg/g ww)	C.V. (%)	conc. soft tiss. / conc. shells
Cd	0.143	28	0.027	90	5.3
Cr	0.307	43	0.394	45	0.8
Cu	1.51	24	1.45	18	1.0
Hg	0.050	32	0.005	48	10.0
Pb	4.22	95	3.97	33	1.1
Zn	25.8	57	5.9	62	4.4

2. Variations in soft tissue metal concentration with mussel size

The statistical data relating metal contents/concentrations in the soft tissues of the mussels to their weight are presented in Table 3.



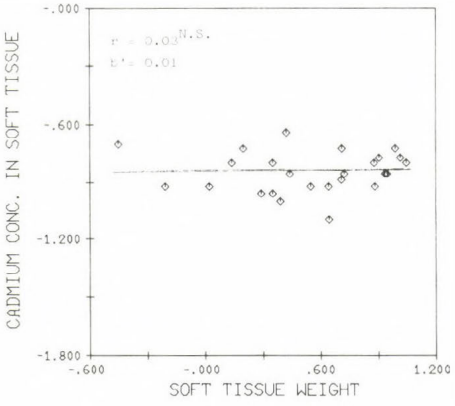
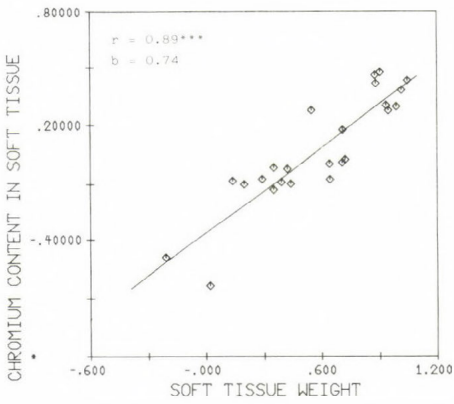


Fig. 1a



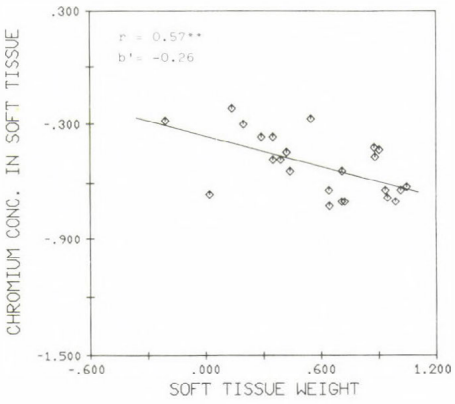
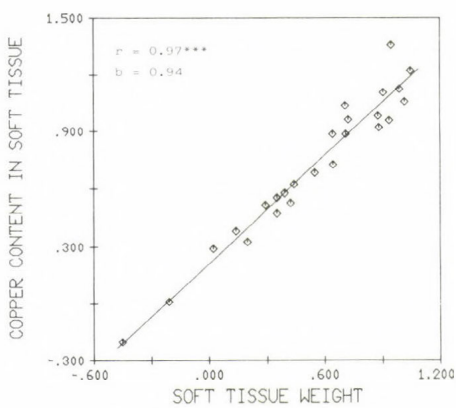


Fig. 1b



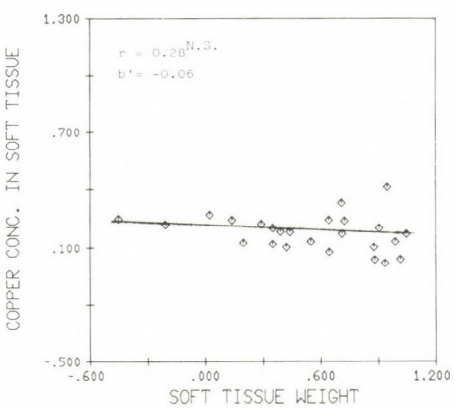
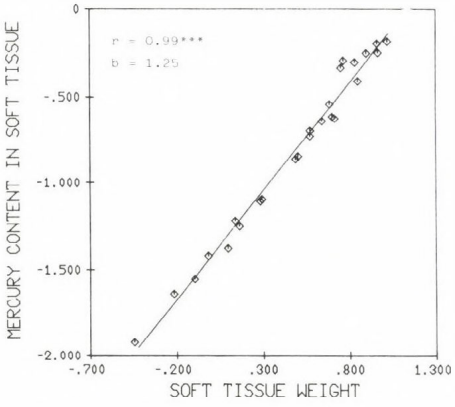


Fig. 1c



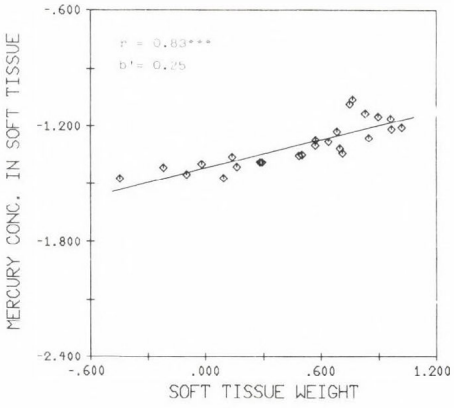
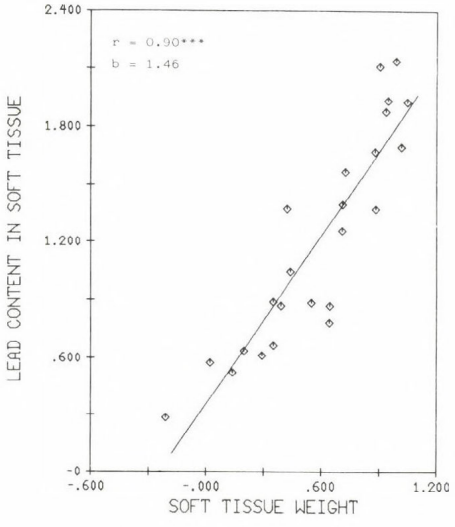


Fig. 1d



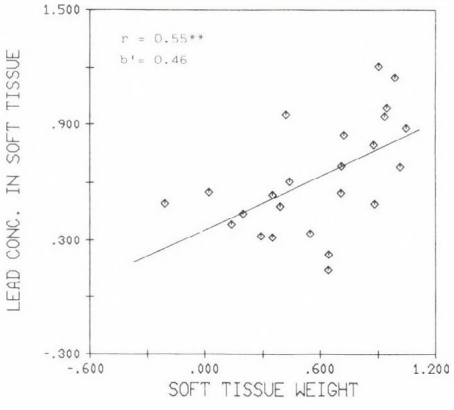
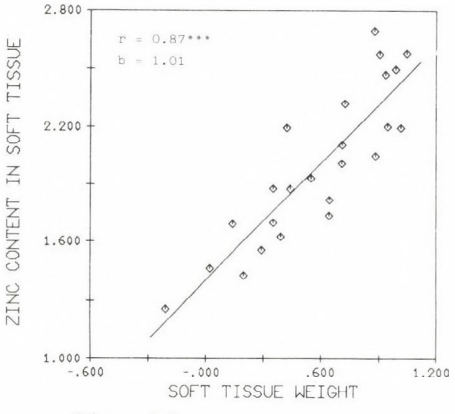


Fig. 1e



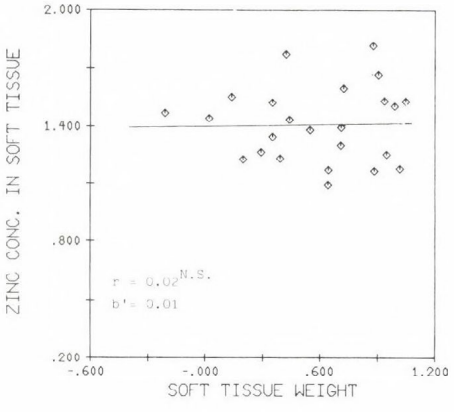


Fig. 1f

Table 3. Statistical information relating metal content (µg) or metal concentration (µg/g wet weight) in the soft tissue to the size (g wet weight of soft tissue) of Mytilus edulis L.. All computations are made on log-transformed data. b = regression coefficient relating metal content to mussel size; b' = regression coefficient relating metal concentration to mussel size (b'=b-1); and 95%-confidence limits of b and b'. Significance levels: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; N.S.: Not significant.

Metal	b	b'(=b-1)	±95%-conf. limits
Cd	1.01 N.S.	0.01 N.S.	0.12
Cr	0.74**	-0.26**	0.16
Cu	0.94 N.S.	-0.06 N.S.	0.10
Hg	1.25***	0.25***	0.07
Pb	1.46**	0.46**	0.31
Zn	1.01 N.S.	0.01 N.S.	0.25

2.1 Cadmium. The cadmium concentrations in the soft tissues of the mussels varied between 0.08 and 0.23 µg Cd/g wet weight. The slope of the regression line relating cadmium concentration to size was 0.01 and was not significantly different from zero (Fig. 1a). The cadmium concentration in the soft tissue therefore was independent of mussel size.

2.2 Chromium. The concentrations of chromium in the soft tissues varied between 0.19 and 0.60 µg Cr/g wet weight. The concentration decreased significantly ($P < 0.01$) with size (Fig. 1b). The slope of the regression line relating Cr-concentration to size was -0.26 (Table 3).

2.3 Copper. The copper concentrations in the soft tissue were independent of mussel size (Fig. 1c). The concentrations in the soft tissues varied between 1.05 and 2.58 µg Cu/g wet weight.

2.4 Mercury. The mercury concentrations in the soft tissue varied between 0.03 and 0.04 µg Hg/g wet weight in the smallest mussels (shell length: 26-35 mm), but were significantly higher

Fig. 1 (the previous two pages): Double logarithmic plot of the relationships between total metal content in soft tissue per individual and soft tissue weight (left), and metal concentration in soft tissue and soft tissue weight (right). (A): Cadmium; (B): Chromium; (C): Copper; (D) Mercury; (E): Lead; (F): Zinc. r= coefficient of correlation; b and b'= regression coefficients (=slope of regression line). Significance levels: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; N.S.: Not significant.

(0.05-0.09 µg Hg/g wet weight) in the larger mussels (shell length: 50-78 mm). The concentration increased significantly ($P < 0.001$) with mussel size (Fig. 1d). The slope of the regression line relating mercury concentration in soft parts to soft tissue weight was 0.25 (Table 3).

2.5 Lead. The mean lead concentration in the mussels was approximately 15 times higher than the background level in the Limfjord. The concentrations in the smallest mussels (shell length: 24-48 mm) were 2-4 µg Pb/g wet weight, whereas the concentrations were up to 16 µg Pb/g wet weight in the larger mussels. The variation was especially great in the largest mussels. The correlation between lead concentration in soft tissue and mussel weight was significant ($P < 0.01$) positive in slope (Table 3). The slope of the regression line was 0.46 (Fig. 1e).

2.6 Zinc. Zinc concentrations in the soft tissues varied between 12 and 65 µg/g wet weight. The correlation between zinc concentration and weight of soft tissue was insignificant, showing that the zinc concentration was independent of mussel size (Fig. 1f).

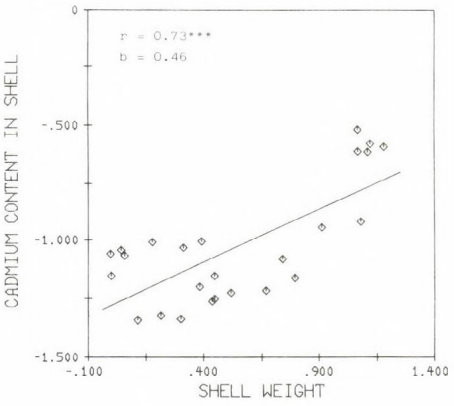
3. Variations in shell metal concentrations with mussel size

The shell length is commonly used as a measure of the size or age of the mussels (Phillips, 1980). The shell weight, however, might be a better estimate of the size/age, owing to the fact, that the thickness of the shells depends on the growth rate. A high growth rate results in very thin shells with a low weight in relation to the shell length, whereas a slow growth rate results in thicker and therefore in relation to shell length, heavier shells. In the present paper the weight of the shells are used as a measure of mussel size. The statistical data relating metal contents/concentrations in the shells of the mussels to the weight of the shells are presented in Table 4.

3.1 Cadmium. The relationship between cadmium in shells and size of the shells seems to be of a curved nature on a double logarithmic plot (Fig. 2a). The cadmium concentration decreased rapidly from approx. 0.09 µg Cd/g wet weight in small mussels (shell length: 24 mm) to approx. 0.02 µg/g in mussels with a shell length of approximately 50 mm. In the larger mussels (shell length: 50-81 mm) the cadmium concentration was rather constant at a level of 0.01-0.02 µg Cd/g wet weight.

The cadmium concentration in the shells decreased significantly ($P < 0.001$) with mussel size (Table 4). The slope of the regression line relating cadmium concentration to the shell weight was -0.56 (Fig. 2a).

3.2 Chromium. The chromium concentration in shells decreased significantly ($P < 0.01$) with mussel size (Table 4). The slope of the regression line relating chromium concentration to shell weight was -0.22. The relationship on the double logarithmic



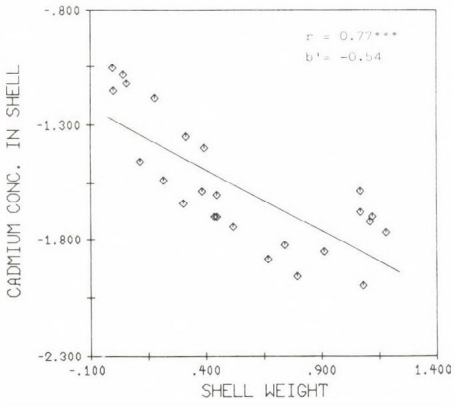
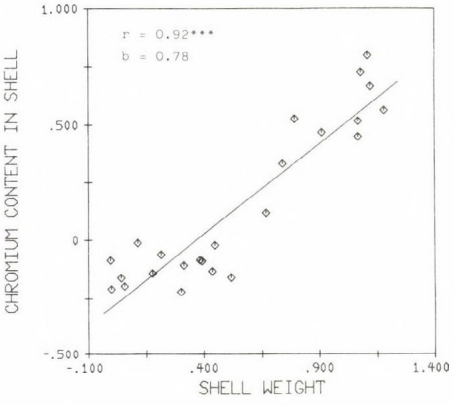


Fig. 2a



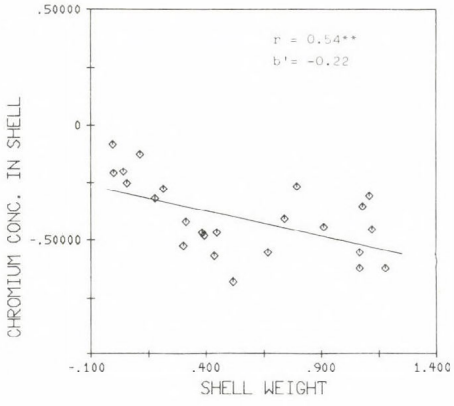
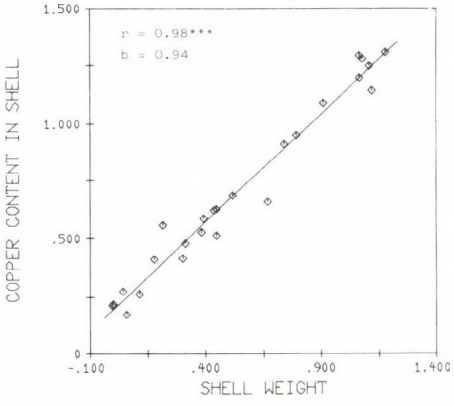


Fig. 2b



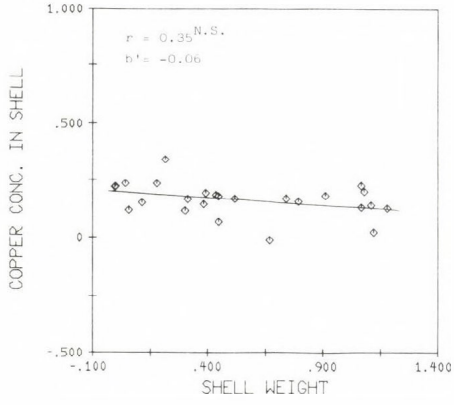
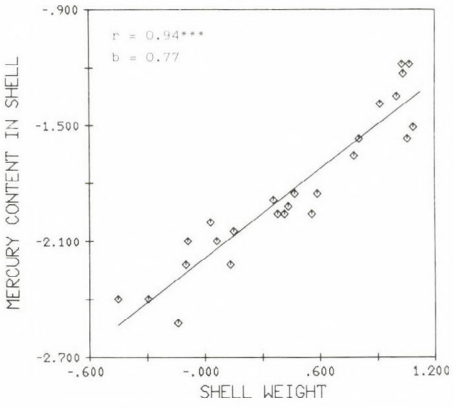


Fig. 2c



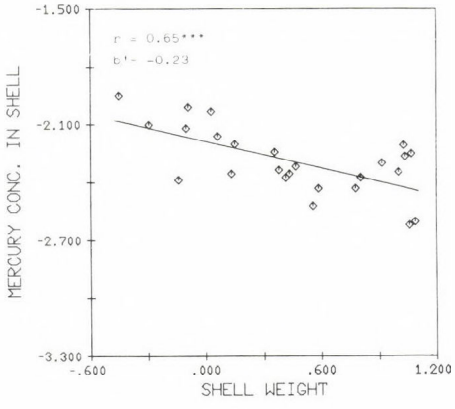
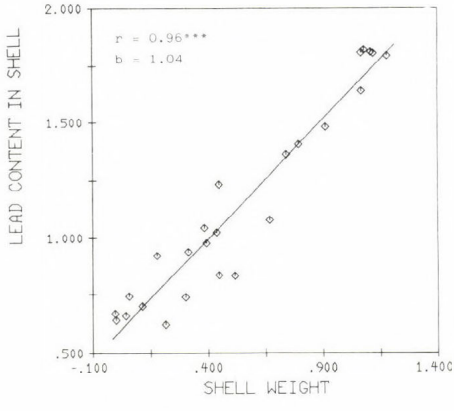


Fig. 2d



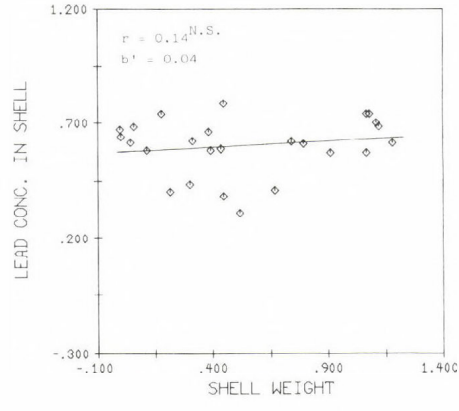
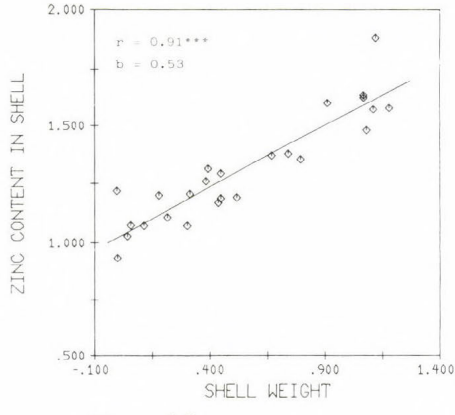


Fig. 2c



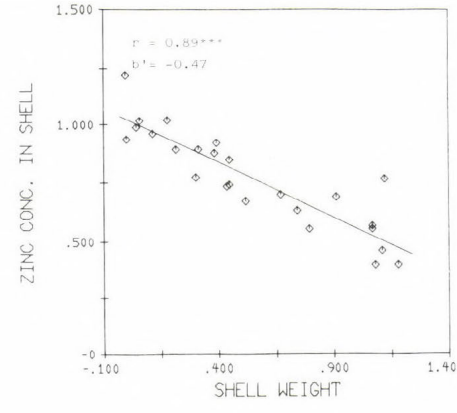


Fig. 2f

Table 4. Statistical information relating metal content (µg) or metal concentration (µg/g wet weight) in the shells to the size (g wet weight of shell) of Mytilus edulis (L.). All computations are made on log-transformed data. b = regression coefficient relating metal content to mussel size; b'= regression coefficient relating metal concentration to mussel size (b'=b-1); and 95%-confidence limits of b and b'. Significance levels: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; N.S.: Not significant.

Metal	b	b'(=b-1)	±95%-conf. limits
Cd	0.46***	-0.54***	0.19
Cr	0.78**	-0.22**	0.15
Cu	0.94 N.S.	-0.06 N.S.	0.07
Hg	0.77***	-0.23***	0.12
Pb	1.04 N.S.	0.04 N.S.	0.13
Zn	0.53***	-0.47***	0.10

plot seems to be of a curved nature (Fig. 2b). The concentration decreased significantly with size in mussels with a shell length up to approximately 50 mm. In the larger mussels (shell length: 50-81 mm) the chromium concentrations were variable with no registrable dependence of mussel size.

3.3 Copper. The copper concentration in shells was independent of mussel size (Table 4). The concentration level in the shells was rather constant 1-2 µg Cu/g wet weight. The slope of the regression line relating copper concentration to shell weight was -0.06, and not significantly different from zero (Fig. 2c).

3.4 Mercury. The correlation between mercury concentration in shells and mussel size was significantly ($P < 0.001$) negatively sloped (Fig. 2d). The slope of the regression line was -0.23 (Table 4). The mercury concentrations in the shells varied between 0.002 and 0.011 µg/g wet weight.

3.5 Lead. Lead concentrations in mussel shells showed no dependence of mussel size (Table 4). The slope of the regression line relating lead concentration to shell weight was 0.04, which was not statistically different from a slope of zero (Fig. 2e). The concentrations varied between 2 and 6 µg Pb/g wet weight.

Fig. 2 (the previous two pages): Double logarithmic plot of the relationships between total metal content in shells per individual and shell weight (left), and metal concentration in shells and shell weight (right). (A): Cadmium; (B) Chromium; (C) Copper; (D) Mercury; (E) Lead; (F) Zinc. r = coefficient of correlation; b and b'= regression coefficient (= slope of regression line). Significance levels: *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; N.S.: Not significant.

3.6 Zinc. Zinc concentration in the shells decreased significantly ($P < 0.001$) with mussel size (Fig. 2f). The slope of the regression line was -0.47 (table 4). The concentrations varied from 8-16 µg Zn/g wet weight in the smallest mussels (shell length: 24-42 mm) to 2-5 µg Zn/g wet weight in the larger mussels (shell length: 50-81 mm).

DISCUSSION

Koide et al. (1982) have suggested, that bivalve shells offer several advantages over soft tissues for the monitoring of heavy metals. Furthermore, in has been proposed, that analysis of ancient shells could function as a "geological record" of the pollution induced changes in metal levels in the environment (Bertine & Goldberg, 1972; Ferrell et al., 1973). There is, however, a lack of information concerning heavy metal uptake and deposition in shells (Phillips, 1980). The metal concentrations in shells of Mytilus edulis are in the present investigation shown to be size-dependent. In addition, the concentration levels of the six metals analyzed are well below the levels in soft tissues, which together with great matrix interferences from calcium give rise to analytical problems. Thus, on basis of the present knowledge, the shells of Mytilus do not seem to be more suitable than soft parts for monitoring purposes.

The size-dependent relationships observed in the present investigation are summarized in Table 5.

Table 5. The observed relationships between metal concentrations in respectively soft tissues and shells of Mytilus edulis and mussel size. b'= slope of regression line relating metal concentration to mussel weight.

	slope (b')	soft tissues	shells
Concentration independent of size	÷0.06 - +0.04	Cd,Cu,Zn	Cu,Pb
Concentration decrease with size	÷0.22 - ÷0.26 ÷0.47 - ÷0.54	Cr -	Cr,Hg Cd,Zn
Concentration increase with size	+0.25 +0.46	Hg Pb	- -

In the literature different kinds of relationships between metal concentration in soft tissues of mussels and body size have been reported. Cossa et al., (1980) reported, that the concentrations of Cd, Cu, Fe, Mn, Ni and Zn in soft tissue of Mytilus edulis all decreased significantly with mussel size. De Wolf (1975) found, that the concentration of mercury either increased with

size or was independent of size. Schulz-Baldes (1973) found, that the concentration of lead decreased with mussel size. Boyden (1974; 1977) found, that the concentrations of Cu, Fe, Mn, Pb, Ni and Zn decreased with size whereas the concentration of Cd was independent of size, and Lobel & Wright (1982) reported, that the zinc concentration was independent of body size as determined by dry weight of the soft parts.

In the mussel Mytilus edulis planulatus (Lamarck) it was found, that the copper concentration decreased with size, that the cadmium and lead concentrations increased with size, and that the zinc concentration was independent of size (Ritz et al., 1982). In addition, Harris et al. (1979) have presented data showing, that the concentrations of cadmium and zinc in this species increased with size, that the concentrations of iron, manganese and copper decreased with size, and that the concentration of zinc was independent of size.

So, in general there is a lack of consistency in the relationships between age or size of Mytilus spp. and the metal concentrations in the soft tissues.

Several processes may be responsible for the observed size-dependent differences in metal concentrations:

(1) A negative correlation between metal concentration and size may arise if the metal uptake by smaller individuals is more rapid than uptake by large individuals. This has in fact been shown to be the case for several metals (Schulz-Baldes, 1974; Phillips, 1976). This explanation may apply for the negative correlation observed for chromium in soft tissues in this investigation.

(2) A positive correlation between metal concentration and size can be attributed to:

- growth dilution, i.e. when tissue is added faster than metal. This is often the case for small individuals, in that the growth rate of younger individuals nearly always exceeds the growth rates in older individuals.

- a net-uptake (accumulation) of metals throughout the lifetime of the mussels. This is often the case for mercury in fish (Cross et al., 1973; Phillips, 1980).

Accumulated metals in Mytilus may be stored as metalloproteins (metallothioneins) or in membrane-limited vesicles and thereby be detoxificated (Nöel-Lambot, 1976; Marshall & Talbot, 1979; George, 1980; Köhler & Riisgård, 1982). These processes are induced by exposure to high metal concentrations (Roesijadi et al., 1982) and may attribute significant to the accumulation of toxic metals with increasing age.

In the present investigation the concentrations of mercury and lead in soft tissues increased significantly with mussel size. The concentration levels of these metals were clearly enhanced in sediment and biota at the sampling location compared to the

background levels in the Limfjord (Brix & Lyngby, 1984). Detoxification of the metals by binding to metallothioneins or precipitation in membrane-limited vesicles may have been responsible for the observed mercury and lead accumulation with age. This is in accordance with information from the literature, which suggests, that correlations are often significant and positively sloped in metal-enriched environments (Strong & Luoma, 1981).

(3) Independence between metal concentration in soft tissue and mussel size occurs when the uptake- and excretion-rates of the metals balance. The equilibration is determined by the balance between uptake and excretion of the metal. Uptake rates will depend upon the level of metal exposure, and the excretion rate will be determined by the physiological exchange of metal in the tissue. Metals with slow exchange rates will be more likely to show positive sloped correlations with body size at lower levels of exposure than will metals with more rapid exchange rates (Strong & Luoma, 1981).

Some authors (Boyden, 1974; 1977; Davies & Pirie, 1980) have suggested, that the slopes of the regression lines are uniform for a given metal and species, and that the intercept of the regression line with the y-axis (equivalent to the concentration in a individual of 1 g) therefore could be used to compare environmental metal levels in different locations. However, several investigations have shown, that the metal concentration-size relationships changes in slope with season, location, metal exposure, etc. (e.g. Phillips, 1976; Cossa et al., 1980; Strong & Luoma, 1981). Because of this inconsistency it is not possible to apply a simple weight normalisation technique, which would effectively eliminate the influence of mussel size on the results of monitoring surveys.

The best way to conduct a monitoring survey using mussels is undoubtedly to collect a large size-range of mussels at each sampling location, and to analyse the mussels individually. This approach, however, demands a very great number of analyses, and will therefore probably not be realistic in greater monitoring surveys. Alternatively, mussels of a limited size-range may be sampled, or only the largest 50% of the mussels may be sampled at each location and analyzed in bulk (Phillips, 1980; Strong & Luoma, 1981). Whatever approach is chosen, it is essential to know the relationship between metal concentration and body size, in order to avoid misinterpretation of the results.

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DISCUSSION

AMIARD-TRIQUET, C: In our laboratory we have carried out a comparable field study in an uncontaminated coastal area. When the dry weight of soft tissues increases to 400-500 mg, the concentration of Cd and Pb in soft tissues decreases. For higher weights, the concentration of lead remains steady whereas the concentration of Cd depends on seasonal factors. Have you used mussels of the same range of size and have you

some explanation for this discrepancy between your results and ours?

BRIX, H: The size of the mussels used in our experiments were of a similar size as those used in your investigation /approx. 0.3-12 g wet weight/. The discrepancy in the observed relationships, at least for lead, in the two investigations is probably caused by the fact that your mussels were collected at an incontaminated location, whereas the mussels used in our investigation were sampled at a location with elevated levels of lead. There might also have been differences in the physical/chemical conditions /salinity, pollution level, etc/ at the sampling locations, which might influence the metal concentration - size relationships.

SALÁNKI, J: You outlined some ways why differences may occur in dependence of size and heavy metal concentration. Do you have any proposal for physiological mechanisms which could be responsible for these differences between ions?

BRIX, H: I think there are several physiological mechanisms which might have influence on these relationships. Firstly, different elements have different exchange rates in the soft tissues of the mussels. Some metals are bound almost irreversibly in the different organs, whereas others are excreted more easily. So, metals with low exchange rates - such as mercury will be more likely to accumulate in the tissue with increasing age, than will metals with rapid exchange rates. Secondly, the detoxification of elements by binding to metallothioneins or precipitation in vesicles occurs in different degree for the different metals. Finally, the uptake and excretion of some metals may be regulated.

HALLEBACH, R: We have data about the same effect for fishes. If we exposed fish to essential elements /Zn, Cu/, then we have not found a correlation to the concentration in the excretion. By the exposure to not essential elements /Hg, Cd/ we found a good correlation in the elimination. I think it is a problem of the homeostasis in the body.

BRIX, H: There are some evidence, that the essential metal copper is regulated in Mytilus, and that Mytilus therefore cannot be used as an indicator organism for copper pollution. In the Ålborg-area in the Limfjord the copper concentrations are significantly elevated in the sediment, in the seagrass Zostera marina and in macroalgae, whereas the concentration levels in Mytilus at the same sampling locations are at the background level. Therefore, the results of the described investigation also indicate that copper is regulated in Mytilus.

WACHS, B: We can agree with the results that in general the metal uptake by young individuals goes more rapidly than by large mussels found in lab-experiments with river fishes.

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TROUTS (*SALMO GAIRDNERI* RICH.) AS BIOINTEGRATORS FOR POLLUTANTS. A NEW METHOD OF RECORDING POLLUTANTS

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SUMMARY

The method detects short-term and intermittent effects of pollutants in running water.

These pollutants are taken up by organisms, e.g. trouts, at any time. The phase shifting in the level of pollutants in the succession for the resorption organ to the elimination organ allows to determine the data of effectiveness, of concentration and of the nature of the pollutant.

Shock caused by heavy metals can still be detected after months. The way of elimination allows to state whether the pollutant has been taken up through the gills or through the intestines.

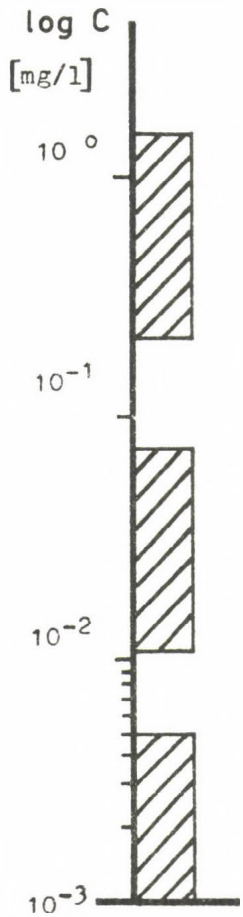
This method lends itself for drinking-water pollution control and for the surveillance of fish farms.

INTRODUCTION

The aim of the investigation was to develop a highly sensitive method seizing pollutants in the range of drinking water limits.

Well-known proposals of applying bioindicators to surface waters are based on using toxicity or sensitivity tests, when death or reactions of behaviour are analysed.

Figure 1. demonstrates the attainable ranges of detection of various methods to bioindication and the required sensitivity of drinking-water control.



1.5 mg/l /Althaus/Jung 1971/

range LC_{50}

0.21 mg/l /WSK 1975/

75 µg/l

range of threshold concentration for sensitivity monitoring /= 5% LC_{50}/

11 µg/l /Morgan 1977/

5 µg/l /WHO/

range of cumulative biological monitoring /drinking water limit, analyt. limit/

1 µg/l

Fig. 1 Ranges of detection to different methods of bioindication

Beside this high sensitivity in the range of subacute effects the pollutants should still be perceptible selectively and the limits of the period of influence should be discernible. For this purpose the bioindicator had to become a biointegrator.

The concept of my approach is based on the following ideas: Organisms resorb from their environment the substances necessary to their metabolism.

The mechanisms of resorption are non-specific, so that pollutants are taken up, too, partially with an affinity higher than necessary for essential substances. In this way high enrichment factors result from a low concentration in the environment. The high sensitivity of the represented method is based on this effect.

The place of pollutant enrichment in various organs of the body is determined by its chemism. Hereby the enrichment is not only bound to one organ but it is liable to a succession of concentration between the organ of resorption, agent of transport, preferred organs of accumulation and organs of excretion with different time-lags. The pollution passes the chain of these organs like a wave with delayed time-lags. That is the point on which the time-integrating effect of my method is based. In this way a pollutant effect is recorded proportional to time in a chain of organs specific to certain substances and passes afterwards the organism according to laws of speed which are mathematically conceivable.

After that the pollutant is eliminated and the chain of organs, comparable to associative memory and record carrier, is receptive again, similar to an automatically erasing tape loop. The interval of passing is determined by the so-called "biologic half-life period" of the pollutant and lies between hours, regarding to some organic compounds, to some months and years, regarding to some heavy metals.

Within this interval of passing the period of influence and the concentration of pollutant can be determined for each moment from a chemical analysis of the relevant organs with the help of the rules of the kinetic reactions.

MATERIAL AND METHODS

The practical corroboration of the theoretical concept took place in a flow stream apparatus /Fig.2/ in which fish /Salmo gairdneri RICH./ were exposed to a solution of $^{203}HgCl_2$ in a concentration of 5 µg/l.

In order to simulate practical conditions, groups of twenty fish were exposed

- for 24 h /short-term exposure/
- for 3 times 24 h, with intervals of 30 days without pollution /intermittent exposure/
- for 13 days /permanent exposure/

Answers to the following questions were expected from the tests:

1. Does the low concentration of pollutants lead to an accumulation in the organs?
2. Is the enrichment in the different organs significantly different and is it evaluable?
3. Does multiple identic exposure lead to cumulating the concentration of the pollutants in the organs?
4. Do the changes of concentrations within the chain of organs lend themselves to retrospective statements concerning the period and the concentration of exposure?

By evaluating autoradiographically microtome sections of complete animals the organs gill, blood, kidney and to a low degree, the liver were detected to be preferred compartments of mercury enrichment.

The used $^{203}HgCl_2$ is taken up to the greater part by the gills into the central compartment, the blood, and distributed there. It is stored in the kidney and in the liver and excreted in a major part by the tubular system /Fig.3/.

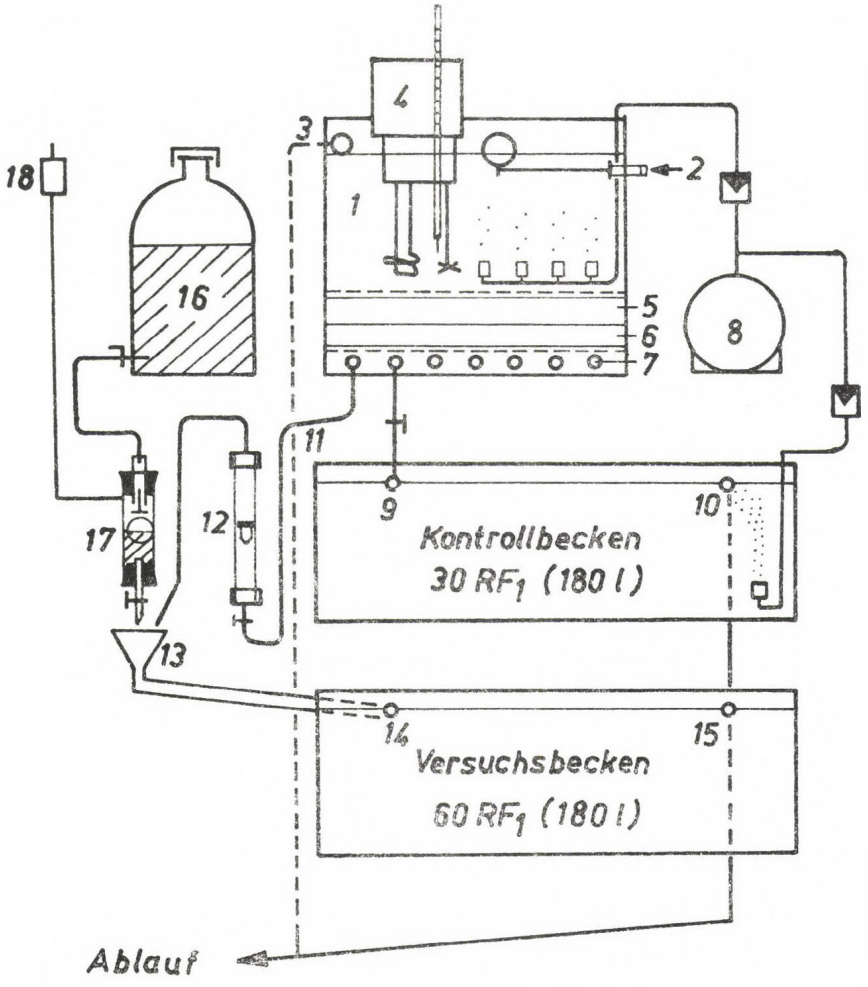


Fig.2 Scheme of the flow stream test lay-out

1. drinking water refining basin
2. drinking water feeding through swimming valve
3. security overflow
4. diving thermostat
5. decarbolith filter
6. charcoal filter
7. drawing prop
8. aeration pump
9. feeding control basin
10. waste-water outlet
11. feeding rotameter
12. flowing through gauge
13. mixing funnel
14. feeding test basin
15. outlet test basin
16. pollutant concentrate
17. dosing device
18. atmospheric pressure compensator

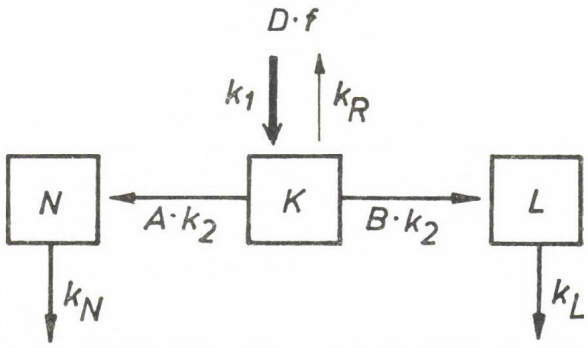


Fig.3 Compartment model of the mercury metabolism

D · f - pollutant load

k_1; k_2; k_R; k_N; k_L - speed constants

K - gill

N - kidney

L - liver

RESULTS

1. Short-term exposure

According to Forth et al. /1977/ and Havlik /1979/, mercury compounds pass easily the membrane of the gills. They are reduced in the erythrocytes and let in the accumulation organs - kidney and liver - in the non-inorganic state. There, the mercury is bound as a metal-thionine complex /Kägi et al. 1981; Cherian and Goyer 1978/ and excreted with delay in an organic bound form via the organs of excretion - kidney and colonic wall /Tohyama et al. 1981; Weiss 1984/.

Twenty-four hours after starting the exposure /E/ the tissue of the gills contained mercury in a 425-fold enrichment compared to the medium. In the following phase the test basin was flown through by uncharged drinking water. Oppositely directed to the exponential decrease of the mercury content in the gills its concentrations in the kidneys increased and tended towards zero after having passed the intersecting point /Fig.4/.

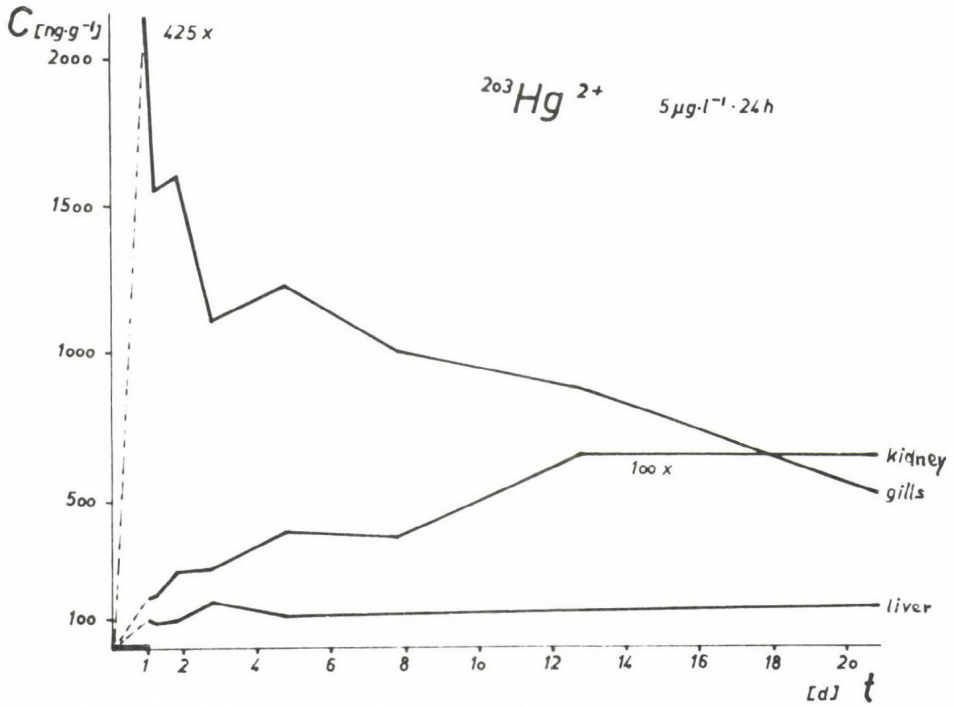


Fig.4 Changes of the mercury concentration in the organs of the trout /Salmo gairdneri RICH./ after a one-day exposure to 5 µg/l $HgCl_2$ in drinking-water

The changes of the mercury concentration in the bioindicator figure in an analogous way after an exposure for 13 days /Fig.5/.

2. Permanent exposure

The results of the long-term exposure are in principle identical to those of the short-term exposure.

The resorption of the pollutants as well as their elimination may be graphed by the exponential function in the general equation

$$y = e^{kx}$$

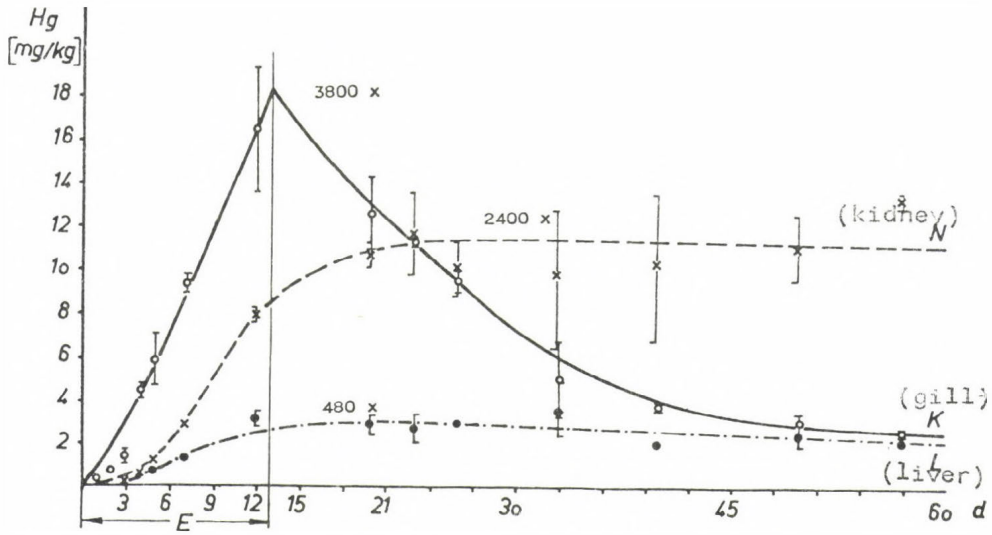


Fig.5 Changes of the mercury concentration in the organs during and after long-term exposure of trouts /Salmo gairdneri RICH./ to 5 µg/l $HgCl_2$ in drinking water

The found mechanisms in the changes of concentration in the investigated succession of organs may be graphed mathematically as a reaction of first order and seem to be applicable to biomonitoring of accumulating pollutants.

The semilogarithmic drawing of the obtained exponential equations results - in compliance with its first derivation - in a mathematical model with straight lines for resorption and elimination /Fig.6/.

The increase of the straight lines $k_x \triangleq \tan \alpha$ is characteristic of each pollutant.

The total process of changing concentration during taking up and elimination of pollutants is reflected in the following function:

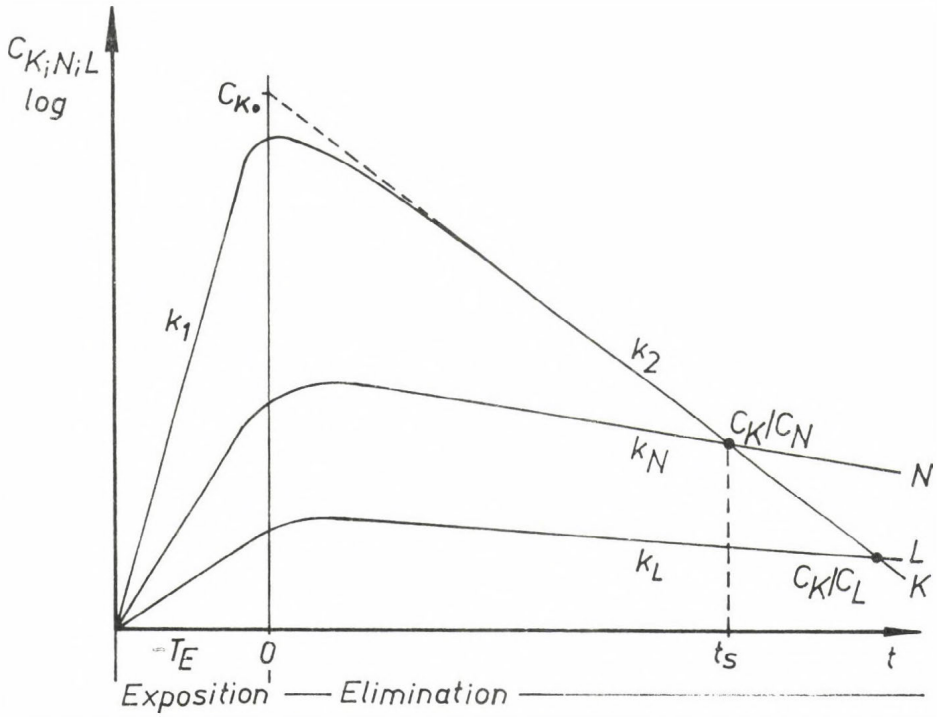


Fig.6 Mathematical model of changes of concentration via chain of organs after a single influence of substance

$$C_K\,/t/ = Df \cdot e^{-k_2 T_E} \int_{-T_E}^{t} e^{-k_2 t}\,dt$$

and

$$C_K\,/t/ = Df \cdot e^{-k_2 T_E} \left[-\frac{1}{k_2} \cdot e^{-k_2 t} \right]_{-T_E}^{t} \quad \text{respectively}$$

C_K - concentration of pollutant in the gill
D - dose /μg/
f - resorption coefficient
k_2 - constant of elimination
T_E - time of pollutant exposure

According to the rules of the kinetics of reactions shown in the generalized representation, the following may be derived from their constant of increase and their intersecting points.

- The maximum of exposure C_{Ko} depending linearly on the charge of pollutants $D \cdot f$ and exponentially on the period of influence T_E.
- The concentration of pollutants in kidney and liver is in proportion to the maximum of exposure.
- The intersecting points C_K/C_N and C_K/C_L follow from the constant time, independent from the starting concentration. By this the maximum of cumulation may be derived.
- C_{Ko} can be calculated from k_2.
- $-T_E$ may be calculated from C_{Ko}.

So it is possible to get information at any time on the influence run down in drinking water plants or fish farms. For this purpose trouts are taken out at equal intervals, their organs are analysed as to expected pollutants and the time and the concentration of the pollutants are determined as shown above.

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DISCUSSION

THEEDE, H: Is it known to you, in what type of chemical speciation the mercury is eliminated by the traut?

HALLEBACH, R: The measures of radioactive $^{203}Hg^{2+}$ cannot answer this question. The opinions in the literature are controversial. Weis, P. /1984/ reported an elimination of 75% in organic-bound or by killifish. I think the proportion may be equal to this.

SVOBODOVÁ, Z: What is your opinion about the food for the fish /trout/ when you test the water for drinking? The food contains fishes as a component and these fishes contain mercury. This amount of mercury is impossible to analyse permanently as a background value.

HALLEBACH, R: The fishes do not take up during the same time natural food /pellet/. Therefore the background is at a low level and constant. Concerning the background of the unpolluted test fishes you must take in account that the level of mercury in the control group does not increase after two years.

SALÁNKI, J: In your model you consider that mercury is taken up through the gills only. However, there are data that substances dissolved in the water are taken up by fishes through the stomach and gut as well. It is not excluded either, that the skin takes part in the uptake mechanism to some degree. So, it may be that your model should be modified in this respect.

HALLEBACH, R: It would complicate my model, but it is yet to be proved.

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TRACE METALS IN EDIBLE CRUSTACEANS FROM LEBANON

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The eastern Mediterranean basin has not yet been extensively investigated with regard to contamination of biota by the various pollutants reported to be existent there. Studies by such organisations as Unesco (1977) which have warned about the serious increase in the volume of waste materials, particularly those containing heavy metals, along the Lebanon-Israel/Palestine coast make it clear that work of this nature is needed. In view of this, and despite the ongoing civil war in Lebanon, a number of pilot studies to determine the levels of heavy metals in certain coastal organisms were undertaken there in the late 1970's. The last of these studies included the analysis of two commercially-important deep-water crustaceans: the large shrimp, Penaeus japonicus Bate, 1888, and the Mediterranean locust lobster, Scyllarides latus Latreille 1803.

COLLECTION AND ANALYSIS

Samples of the shrimp, Penaeus japonicus (average length from rostrum to telson: 154.2mm) were taken from fifteen locations between Tripoli and Tyre, Lebanon, 800-1000m offshore and at an average depth of 35m. One to three shrimp comprised each sample. Samples of the lobster, Scyllarides latus (average length: 252.5mm), were collected at eight locations between Tripoli and Tyre at an average depth of 25m, about 400m from shore. One lobster comprised each sample. A map of the collecting sites is shown in Fig. 1.

After the viscera of all samples were removed and discarded the edible muscle tissue and exoskeletal tissue were separated, rinsed in distilled water, and stored in plastic containers in a freezer at -5 °C to -2 °C until processing was possible. All utensils and containers were pre-soaked overnight in hot water and a good detergent according to the FAO recommended procedure (Bernhard, 1976) and then rinsed in distilled water.

Prior to wet-ashing, the samples were thawed and rinsed again briefly in distilled water. They were then weighed,

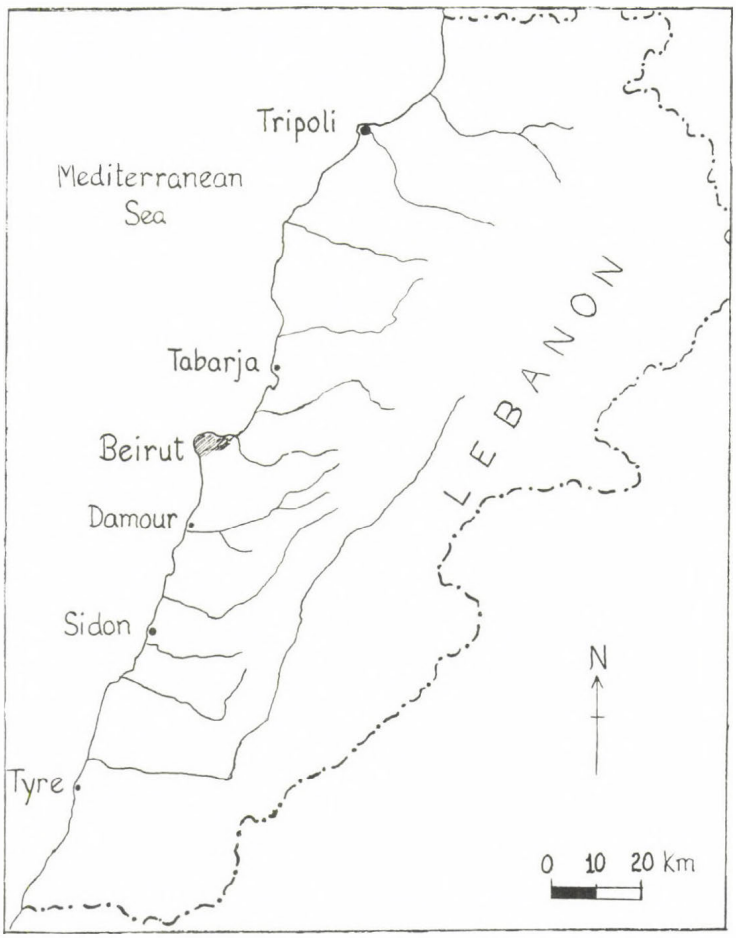

Fig. 1. The coast of Lebanon from which samples of shrimp and lobster were collected and analysed for heavy metals.

oven-dried at 60 °C to 70 °C for 24-48 hours, weighed again, then ground to powder with an agate and mortar pestle. Subsamples of 0.5g each were mixed in 150ml "Pyrex" flasks with 10ml nitric-perchloric acid (3:1 by volume), dried at 150 °C for 12-24 hours or until no moisture remained, then brought to volume with 75ml of HCl. Blanks were processed in the same manner.

Levels of Pb, Cd, Cu, Ni, Fe, Zn and Cr were determined in each sample using a Perkin-Elmer Model 306 atomic absorption spectrophotometer at the Purdue University School of Civil Engineering (Environmental Engineering Department) in Indiana, U.S.A., using a flame with a lean mixture of air and acetylene. Standards were made up of 1 + 1 HNO_3. Maximum and minimum limits of detectability were as follows:

Element	Maximum	Minimum
Pb	20	0.25
Cd	1	0.01
Cu	5	0.025
Ni	5	0.0625
Fe	50	0.625
Zn	1	0.01
Cr	2	0.03

Background corrections were not performed.

The Tyre samples of shrimp were analysed for Pb, Cu, Fe and Zn levels on a Varian-Techtron AA-5 spectrophotometer with an air-acetylene flame, at the Department of Chemistry, University of Birmingham, Birmingham, England.

Values from each analytical instrument were given in mg/l and then converted to both $\mu g/g$ wet weight and $\mu g/g$ dry weight. The values reported here are in $\mu g/g$ dry weight only.

RESULTS AND DISCUSSION

The metal levels found in the shrimp, Penaeus japonicus, are shown in Table 1 and those found in the lobster, Scyllarides latus, are in Table 2.

Shrimp:

Lead and cadmium levels in the shrimp were relatively stable with a few elevated readings in each tissue at different locations. For the most part, copper was somewhat higher in the shrimp muscle than exoskeleton, except at Beirut (samples #5 and #6), Damour (#7 and #8), and Sidon (#12) in which the exoskeleton concentrated the higher levels. Nickel was quite variable, although the higher concentrations seemed to occur mainly in the exoskeleton . Iron was also very variable in both muscle and exoskeleton of the shrimp and zinc was consistently higher in the shrimp muscle. Chromium was slightly variable.

TABLE 1

Metal concentrations ($\mu g/g$ dry) in samples of muscle (m) and exoskeleton (e) of the large shrimp, Penaeus japonicus, taken from 15 locations along the Mediterranean coast of Lebanon. The number of specimens comprising each sample analysed appear in parentheses.

Sample # and Location	Tissue	Pb	Cd	Cu	Ni	Fe	Zn	Cr
Tripoli								
1 (3)	m	7.2	0.8	56.3	9.4	14.1	47.3	2.2
	e	22.5	1.6	26.3	14.1	107.8	6.4	4.5
2 (3)	m	7.5	0.7	52.5	nd*	220.3	62.0	11.6
	e	7.0	0.4	31.0	9.4	223.0	nd	6.9
Tabarja								
3 (3)	m	8.0	0.9	41.9	9.2	182.8	78.5	1.6
	e	22.5	1.0	34.7	13.2	192.2	5.0	9.2
4 (3)	m	7.8	1.0	56.0	4.7	220.4	131.7	2.2
	e	20.5	1.2	35.0	8.9	183.0	20.3	4.7
Beirut								
5 (3)	m	23.0	1.6	68.5	4.7	304.7	134.3	4.4
	e	7.5	1.2	89.1	nd	267.2	47.7	9.4
6 (3)	m	7.8	0.8	51.2	nd	154.7	54.8	2.0
	e	8.2	1.4	60.1	14.1	164.1	36.1	4.5
Damour								
7 (2)	m	8.1	0.9	28.1	4.7	14.1	54.9	6.8
	e	7.6	0.7	62.5	4.2	118.0	6.9	1.7
8 (3)	m	21.5	1.0	39.4	nd	117.2	80.7	7.0
	e	7.6	0.8	60.6	9.4	482.8	17.7	2.0
Sidon								
9 (3)	m	7.0	1.3	53.5	nd	492.2	41.0	4.5
	e	23.5	1.6	27.2	9.0	164.1	10.2	1.8
10 (3)	m	7.5	1.1	43.2	nd	98.8	57.8	1.8
	e	7.3	1.5	29.1	14.1	117.2	12.2	2.0
11 (2)	m	7.2	0.8	28.1	nd	182.0	92.3	11.8
	e	7.4	1.0	11.3	18.8	14.5	7.6	1.5
12 (2)	m	7.1	0.6	20.6	nd	107.8	44.3	1.9
	e	8.3	1.3	24.4	nd	89.1	15.8	2.6

TABLE 1 Continued

Sample # and Location		Tissue	Pb	Cd	Cu	Ni	Fe	Zn	Cr
Tyre									
13	(1)	m	nd	-	1.9	-	111.6	62.3	-
		e	nd	-	2.0	-	271.6	2.3	-
14	(1)	m	nd	-	16.9	-	291.6	107.3	-
		e	nd	-	1.9	-	112.0	32.3	-
15	(1)	m	nd	-	17.0	-	171.6	47.3	-
		e	nd	-	1.8	-	156.6	17.3	-

*not detected

Lobster:

Lead and cadmium readings in the lobster were fairly uniform and similar to those of the shrimp. There was no significant difference between muscle and exoskeleton concentrations of either metal, although cadmium was slightly elevated in a few samples of exoskeleton (#5 at Sidon, #8 at Tyre) and muscle (#4 at Beirut). Copper seemed generally lower in the lobster than in the shrimp and there was little difference between muscle and exoskeleton copper levels in most of the samples. However, the lobster muscle from Beirut had considerably higher copper than any of the other samples. Nickel was variable, with comparatively high readings in both the muscle and exoskeleton of sample #8 from Tyre. Iron was also variable, but it was clearly more highly concentrated in the exoskeletal tissue of each lobster analysed, except for sample #8 from Tyre. As in the shrimp, zinc proved to be highest in most muscle samples of the lobster, the exception again being sample #8 from Tyre. Chromium was slightly variable with relatively higher values in sample #8 from Tyre.

It is interesting to note that, generally, these two crustaceans had very similar metal readings. This is shown quite distinctly in Table 3. Except for nickel and zinc, metal levels in the shrimp muscle and exoskeleton were nearly identical. The lobster muscle and exoskeleton had similar levels of lead, cadmium, nickel and, to a lesser extent, chromium. Bertine and Goldberg (1972) mentioned the possibility that certain metals may be more highly concentrated in the exoskeletons of crustaceans. In the present study, the lobster exoskeleton had higher iron levels than the muscle, and many samples of the shrimp exoskeleton had somewhat higher nickel

TABLE 2

Metal concentrations ($\mu g/g$ dry) in samples of muscle (m) and exoskeleton (e) of the Mediterranean locust lobster, Scyllarides latus, collected from eight locations on the coast of Lebanon. One specimen comprised each sample.

Sample # and Location	Tissue	Pb	Cd	Cu	Ni	Fe	Zn	Cr
Tripoli								
1	m	7.0	0.7	24.4	9.5	32.9	38.7	2.2
	e	7.5	0.6	20.6	nd*	145.3	nd	2.4
2	m	6.0	0.4	29.1	9.4	70.4	77.4	2.0
	e	7.8	0.4	24.6	nd	351.6	3.1	6.9
Beirut								
3	m	6.5	0.8	67.5	nd	51.6	92.3	1.8
	e	6.8	0.8	24.8	9.8	389.1	3.2	2.8
4	m	7.2	2.3	75.0	nd	89.1	72.1	2.0
	e	7.5	0.7	7.8	nd	182.8	nd	2.3
Sidon								
5	m	7.1	0.5	28.1	18.8	182.0	87.8	6.9
	e	7.3	2.3	33.9	9.6	482.8	10.6	2.1
6	m	7.6	0.3	28.3	9.4	89.1	91.6	2.1
	e	7.5	0.5	33.8	nd	483.0	29.3	6.9
Tyre								
7	m	6.0	0.7	41.3	nd	52.0	54.1	2.0
	e	5.8	0.6	28.1	9.3	426.6	17.3	11.8
8	m	7.8	0.8	37.5	26.3	389.0	20.3	11.6
	e	7.0	2.3	45.0	58.1	107.8	54.1	16.3

*not detected

than the muscle. On the other hand, zinc was definitely more highly concentrated in the lobster and shrimp muscle. It would be difficult to determine from the present data, however, whether or not the exoskeletons of these crustaceans actually have an affinity for iron or nickel since their readings were highly variable in both species.

TABLE 3

Overall ranges and average concentrations (μg/g dry) of heavy metals in samples of shrimp and lobster muscle (m) and exoskeleton (e) collected from the Mediterranean coast of Lebanon. (nd: not detected)

Pb	Cd	Cu	Ni	Fe	Zn	Cr
			Penaeus japonicus (shrimp)			
			(m)			
nd-23.0	0.6-1.6	1.9-68.5	nd-9.4	14.1-492.2	41.0-134.3	1.6-11.8
8.0	1.0	38.3	2.7	178.9	73.1	4.8
			(e)			
nd-23.5	0.4-1.6	1.8-69.1	nd-18.8	14.5-482.8	2.3-47.7	1.5-9.4
10.0	1.1	33.1	9.6	172.5	15.8	4.2
			Scyllarides latus (lobster)			
			(m)			
6.0-7.8	0.4-2.3	24.4-75.0	nd-26.3	32.9-389.0	20.3-92.3	2.0-11.6
6.9	0.8	41.4	9.2	119.5	66.8	3.8
			(e)			
5.8-7.8	0.4-2.3	7.5-45.0	nd-45.0	107.8-483.0	nd-54.1	1.8-16.3
7.2	1.0	27.3	10.9	321.1	14.7	6.5

It is difficult to assess the significance of the metal levels found in these crustaceans since so few comparison data are available and larger sample numbers need to be examined. One of the few Mediterranean studies of this nature was done by Fukai and Broquet (1965) who studied the iron and chromium content of a number of marine organisms, including shrimp (Crangon crangon and Parapenaeus longirostris) and crabs (Pisa nodipes, Carcinus maenus, and Eriphia verrucosa), from the coasts of France and Monaco. The shrimp they analysed had low iron and chromium in the soft tissue when compared to levels found in the muscle tissue of the species studied here. Their carapaces, however, had levels similar to those of the shrimp exoskeletons in the present study. The soft parts of the crabs which Fukai and Broquet analysed had higher iron, but lower chromium than the edible lobster and shrimp from Lebanon.

Shore crabs, Eriphia verrucosa and Pachygrapsus transversus, collected from the coast of Beirut (Lebanon) in an earlier study (Shiber, 1977) had generally higher metal levels than both the shrimp and lobster samples taken from Beirut in this study. Similarly, samples of prawns, Palaemon elegans, collected from the same area (Shiber, 1979) had higher metal levels, particularly of copper. However, the crabs and prawns in those previous studies were processed whole. Moreover, they inhabit the intertidal zone and are regularly subjected to land-based environmental factors such as exhaust from heavy city traffic, direct contact with raw sewage and garbage, etc..., which could account for higher metal availability and consequent uptake. Although it is doubtful that the shrimp and lobster are totally excluded from being affected by these factors because they live in deeper water and further from the shore, it has been shown in a number of studies (Preston, 1973; Papakostidis et al, 1974; Montgomery and Price, 1979) that biological samples taken from areas of sewage and industrial waste input usually have higher metal levels. Cephalopod molluscs taken from the coast of Lebanon in another study (Shiber, 1981) showed higher metal levels in the Beirut area which is densely populated and has a greater volume of waste products entering the sea than the other collecting sites. However, the Beirut samples of lobster and shrimp, did not have significantly higher metal concentrations than samples taken at the other locations.

The numerous possible sources of metals in the coastal environment of Lebanon have been discussed in some detail in previous studies (Shiber, 1979, 1981; Shiber and Shatila, 1978, 1979). All of Lebanon's sewage is untreated and, along with other waste products from industry and agriculture, enter the sea either directly or indirectly through rivers and streams which terminate at the Mediterranean. At least 15 major rivers enter the sea from the 280-kilometer-long coast of Lebanon. Unfortunately, little is known about the nature of industrial waste in Lebanon or what agricultural products are now being used in the country, which is heavily farmed. Since the civil war began in 1975, government control on the types of pesticides

imported to the country has been very lax, if it has existed at all. Among the brand names of pesticides known to have been used in the past in Lebanon and are most likely still in use at the present time include the following: Aldrin, Heptachlor, B.H.C., Malathion, Parathion, DeMol, Phosphorno, 605, Lindane, Dimecron, Lannate, Rogor, Metasystox, Winter Oil, Summer Oil, Dinitro-Ortho-Cresop (insecticides); Zineb, Maneb, Ferbani, Mangozeb, Karathane, Milcurb, Benlate, Topsin (fungicides); Kelthane, Delnav (acaricides); Gramoxone, Karmex, Hyvar-X-2, Weeder, 4,5T, Uratex, 2,4D (weedicides); Nemagon, Nematam (nematocides) (Shiber, 1979). What has been added to this list in the last few years has yet to be determined. Unesco (1977) reported a significant increase in the use of organo-phosphorous and carbonate insecticides and input of chlorinated hydrocarbons to the Mediterranean along the coast of Israel/Palestine and Lebanon. This could be a contributing factor in increased metal availability to coastal biota.

Tanker traffic to and from the several oil pipeline terminals on the eastern Mediterranean coast might be another source of trace metals here. Also, open-air incineration in Lebanon is common and much of the material burned is made up of petroleum derivatives, which could add to the atmospheric load of particulate metals such as lead and cadmium. Moreover, to what degree the civil war has added to the problem of heavy metals in the environment here, considering the voluminous shelling, burning of oil tanks, use of various types of ammunition, etc..., which has gone on for the past nine or ten years, is open for conjecture, but might be an additional and interesting source of trace metals to be investigated.

Of course, the natural contribution from the geophysical aspect must be considered since the country is undergoing increased erosion. Furthermore, it is possible and has been mentioned before (Shiber, 1981; Shiber and Shatila, 1979) that contaminants could be coming from other areas of the Mediterranean, since the surface currents seem to move in an easterly direction in summer (In: Ahmad, 1972; Schmidt-Nielson, In: LaCombe, 1975).

More work of this nature must be undertaken in Lebanon and in the eastern Mediterranean basin as a whole before any conclusions can be made on the data presented here. It would appear, however, that the metal levels in these and other coastal biota studied recently in this area (Shiber, 1977, 1979, 1980a, 1980b, 1981; Shiber and Shatila, 1978, 1979) are high enough to warrant further study. Although the FAO oulined which species should be used for pollution studies in order to standardize the work in the Mediterranean (Bernhard, 1976), many of these species are not readily available in the eastern basin or are so rare that their use as pollution-monitors would be futile. This is especially so when one considers that abundance has been one of the important factors used in determining which species can be useful pollution indicators. It may be necessary, therefore, to compile an additional, separate

list of possible indicator species for the eastern Mediterranean region. In order to facilitate this, the occurrence, abundance and diversity of intertidal organisms from Lebanon should be studied in some detail and the results compiled with other such data from the remaining countries of the eastern basin.

Furthermore, it would be helpful if future studies include the determination of the volume and nature of waste products entering the sea from Lebanon, and the scrutinization of waste disposal practices of its industries. Likewise, a rigorous up-dating of the list of agricultural products, mainly pesticides, used in Lebanon on a yearly basis is needed. Such a list should include the volume of each product and a detailed listing of its constituents.

When the situation in Lebanon becomes safer (in the near future, it is hoped), more encompassing work ought to be undertaken to investigate trace metals in the coastal biota here, using this pilot study as a point of reference.

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FUNCTIONAL ASPECTS OF HEAVY METAL POLLUTION

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THE TISSUE ACCUMULATION OF HEAVY METALS AND THEIR INFLUENCE ON THE BIOSYNTHESIS IN THE FISH ORGANISM

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The heavy metal ion concentration is constantly increasing under the conditions of rising anthropogenic ecological influence on aquatic ecosystems in different reservoirs, including those used for pisciculture. It is well known that in biotic concentrations these ions activate certain zymotic systems which participate in metabolic synthesis of the organic compounds. They also play a major role in other vital physiological and biochemical processes in both higher and lower vertebrates.

However, high concentrations of heavy metals can considerably change the intensity and direction of metabolism in hydrobionts. As a rule, it is accompanied by metabolic disturbances and the lower growth rate of aquatic animals, including fishes. It calls for a detailed investigation into the mechanism of the physiological impact of different concentrations of the microelements of water habitats upon certain aspects of metabolism in fishes and other hydrobionts.

All these considered, we studied the dependence of the biosynthetic tendency of metabolism in fishes upon different concentrations of the heavy metal ions /manganese, zinc, cobalt and copper/ in water. For this purpose we put onetime portions of metals into the aquariums in concentrations exceeding the background ones by 0.05; 0.5; and 1.0 mg/l for manganese /0.006 mg/l/; 0.02; 0.1; and 1.0 mg/l for zinc /0.003 mg/l/; 0.01; and 0.1 for cobalt /0.01 mg/l/; and 0.01; 0.1; and 1.0 mg/l for copper /0.01 mg/l/ (in terms of cation).

Using radioisotope assay we asserted that both the intensity and direction of biosynthesis in fishes exposed to various concentrations of heavy metal ions in water are determined by their own concentration, duration of the impact upon the organism, the level of their accumulation in organs and tissues, as well as by the specificity and physical and chemical properties of the element itself.

Of all studied metals copper most explicitly inhibited the incorporation of radiocarbon from acetate-2-^{14}C into the albumin synthesized by the liver of the carp. We determined that both the level of metal accumulation in the organs and tissues of fishes and the retardation influence on the synthesis of albumin in the liver went up with the increase of metal concentration in the water /Fig.1,2/.

It should be noted that in the course of the 7-day adaptation of fishes to the copper load different concentrations of metal in water produced different biological effects in the case of biosynthesis in an organism. E.g., at lower /0.01 and 0.1 mg/l/ concentrations of metal in water we observed gradual increase in its accumulation in the liver and higher inhibition of albumin synthesis the longer this metal affected the organism.

The overfeeding of fish organisms with copper /1.0 mg/l/ switches on certain homeostatic mechanisms which help to excrete the exceeding amounts of metal and to restore the functions of the liver. But when the concentration of copper exceeded the background one by 1.0 mg/l, we often observed the death of fishes accompanied by forced respiratory movements, disruption in coordination of movements, and turnovers.

It also should be noted that if the copper content in water goes on to rise we observe, after partial evacuation of metal from an organism, the recurring accumulation of metal in organs and tissues accompanied by the corresponding changes in the biosynthetic directness of metabolism in the organism. So, the

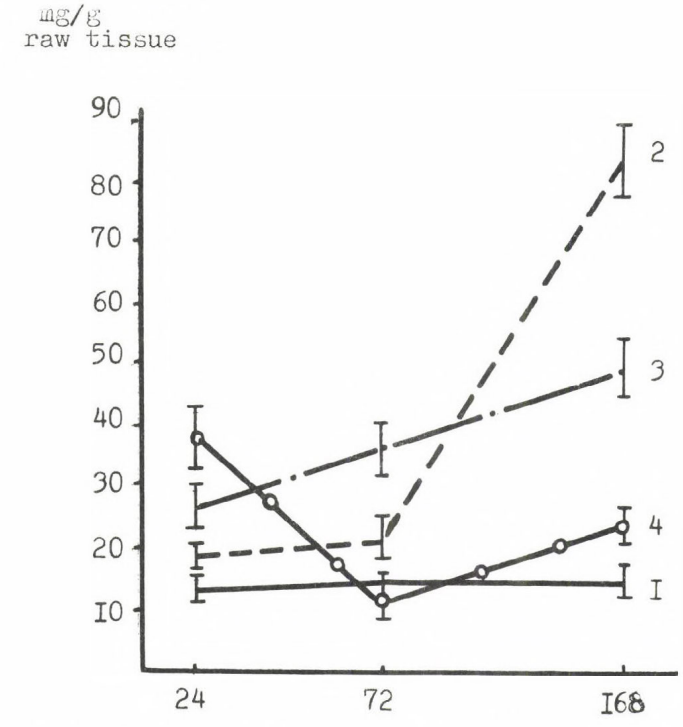

Fig.1 The tissue accumulation of copper in the liver of a carp depending on the metal concentration in water: 1-0.01 mg/l Cu; 2-0.02 mg/l Cu; 3-0.1 mg/l Cu; 4-1.0 mg/l Cu.

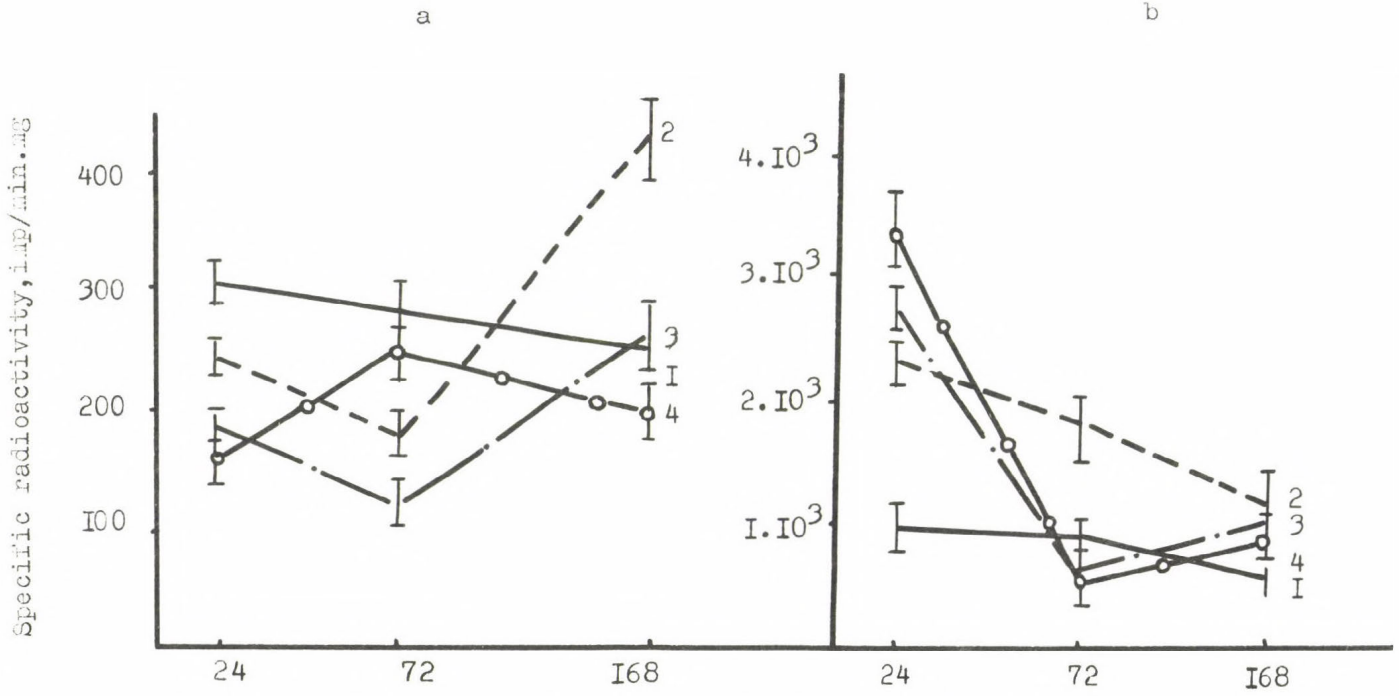

Fig.2 The rate of radiocarbon consumption from acetate-2-^{14}C by the general proteins /a/ and lipids /b/ in the liver of a carp depending on the concentration of copper in water: 1-0.01 mg/l Cu/control/; 2-0.02 mg/l Cu; 3-0.1 mg/l Cu; 4-1.0 mg/l Cu.

fluctuant changes in the intensity of biosynthesis in the organism are caused in the first place by the changes in the metal accumulation levels in organs and tissues of fishes, especially in the liver, during their adaptation to the ion load.

During the 7-day adaptation of fishes to copper contained in the water in biotic /0.01 mg/l/ concentrations we observed not only the restoration of the protein-synthesizing function, but the activating impact of metal on protein synthesis as well.

Due to the effect of the boosted copper concentrations in water the indexes of the protein synthesis intensity in the fish organism also tended to control data which indicates the restoration of the functions of the liver.

The experimental data show that protein-synthesizing system in fishes is extremely sensitive to the boosted copper content in water.

The toxic effect of copper is less prominent in the case of the synthesis of lipids in the liver of fishes. The experimental data show that different concentrations of copper in water substantially increased the carboxyl-tagged sodium acetate consumption in the synthesis of lipids. However, at certain stages of adaptation /72 h/ of carps to the concentrations of metal in water, especially to the boosted ones /0.1 and 1.0 mg/l/, the intensity of inclusion of the acetate-2-^{14}C-based tags into the general lipids extracted from the liver tended to decrease, which proves its excessive accumulation in the organs and tissues and lower effectiveness of the activating influence on the lipid-synthesizing function of the liver /Fig.2/.

During the 7-day adaptation of carps to different concentrations of copper in water the indexes of the intensity of lipogenesis in organism approached the control level.

Thus, the same concentrations of copper in water produced varietal biological impact upon different aspects of metabolism in the organism of fishes.

The copper-initiated failure of protein-synthesizing and change of lipogenic function of the liver in fishes at certain

stages of their adaptation to the copper load were accompanied by the lower consumption of the carboxyl-tagged acetate for the macromolecular complexing secreted by the liver as a bile component. We also observed the decrease of the bile-acid concentration and of their lipid complex in bladder bile which supports the evidence that copper inhibits both the biosynthetic and bile-producing function of the liver /Fig.3/.

We have already mentioned that during the adaptation of fishes to the copper-loads the biosynthesis in the organism is being restored. It can be illustrated by the fact that following the 12-day adaptation of carps to different concentrations of metal in water there occurs a marked tendency of the albumin total to increase in the liver /Fig.4/.

However, under the same conditions the summary lipids in this organ decrease with the simultaneous increase of their content in the muscle tissue /Fig.5/.

Our data show that the boosted concentrations of copper in the organs and tissues produce a marked lipotropic effect, one of its mechanisms being based on the participation of metal in the intertissue and interorgan disproportionation of lipids.

With the increase of copper concentration in water and liver we observed the increase of the percentage of albumins and ceruloplasmins in blood plasma, as well as posttransferrins, compensated by the lower level of transferrins, α_2-macroglobulins and gamma globulins in the same blood plasma.

We also observed the rise of albumin and globulin coefficient under the influence of high concentrations of copper ions in water.

The increase of copper concentration in the water was accompanied by the considerable increase of the chylomicron content in the blood serum compensated by the decreasing amount of LPVP.

So far, the experimental data show that, depending upon the level of its accumulation in organs and tissues of fishes, copper either inhibits or activates the biosynthesis in the organism. The level of metal accumulation in the organs and

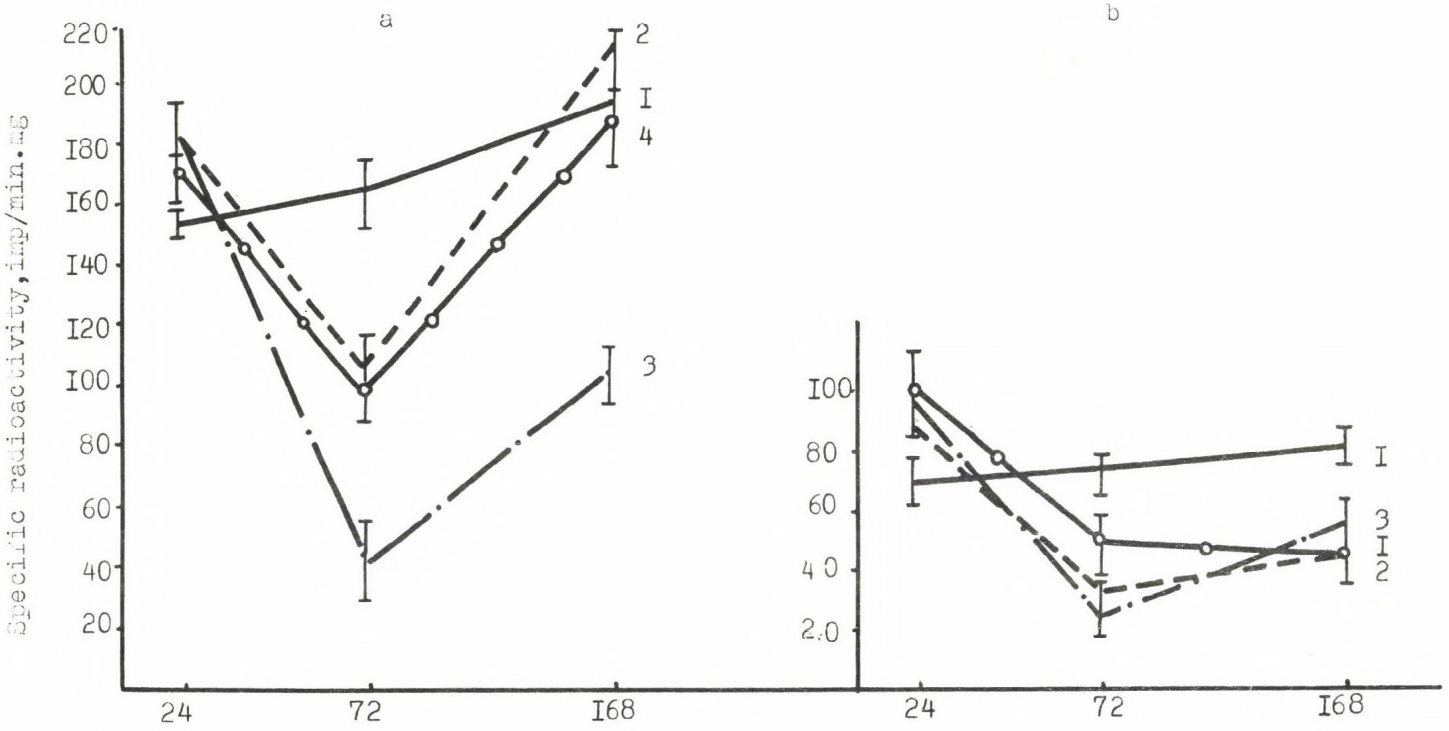

Fig.3 The influence of different concentrations of copper in water on the rate of consumption of the acetate-2-^{14}C-derived tag by the organic components of the bladder bile in fishes: 1-0.01 mg/l Cu; 2-0.02 mg/l Cu; 3-0.1 mg/l Cu; 4-1.0 mg/l Cu.

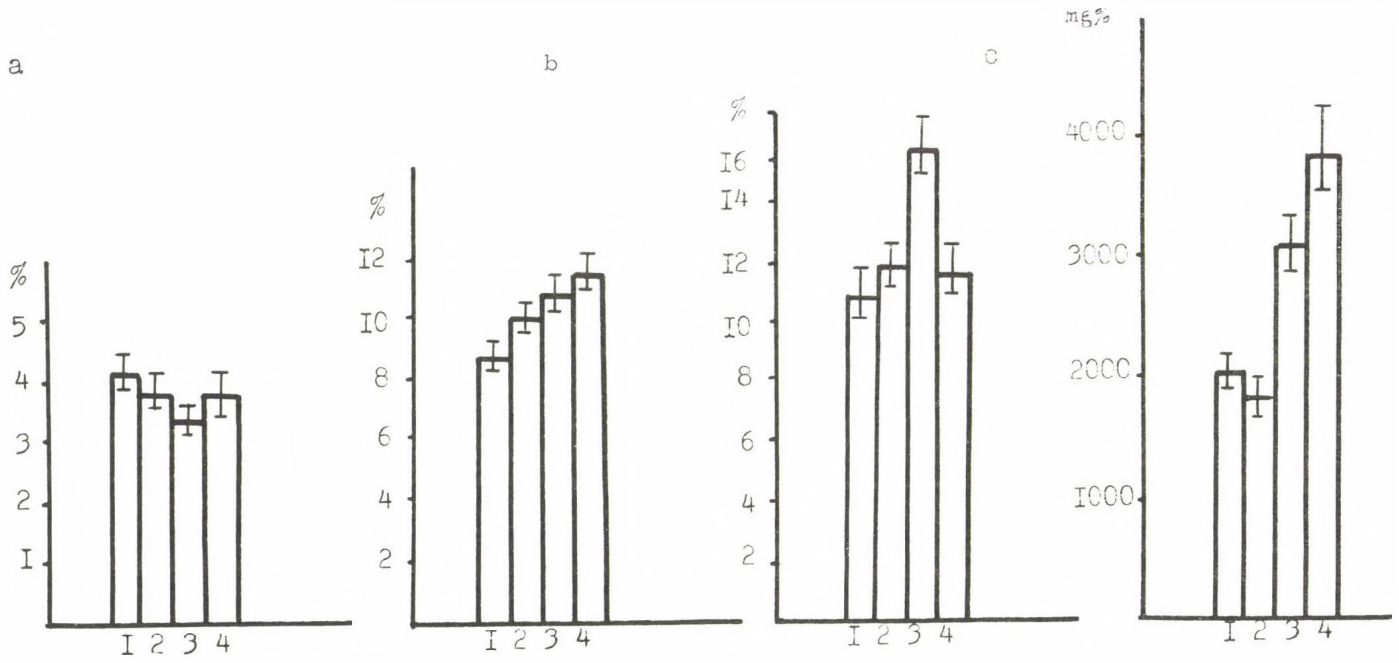

Fig. 4 The general albumin /a,b,c/ and glycogen /d/ content in organs and tissues of a carp depending on copper concentration in water: a-blood; b-liver; c-white skeletal muscles; d-liver. 1-0.01 mg/l Cu; 2-0.02 mg/l Cu; 3-0.1 mg/l Cu; 4-1.0 mg/l Cu

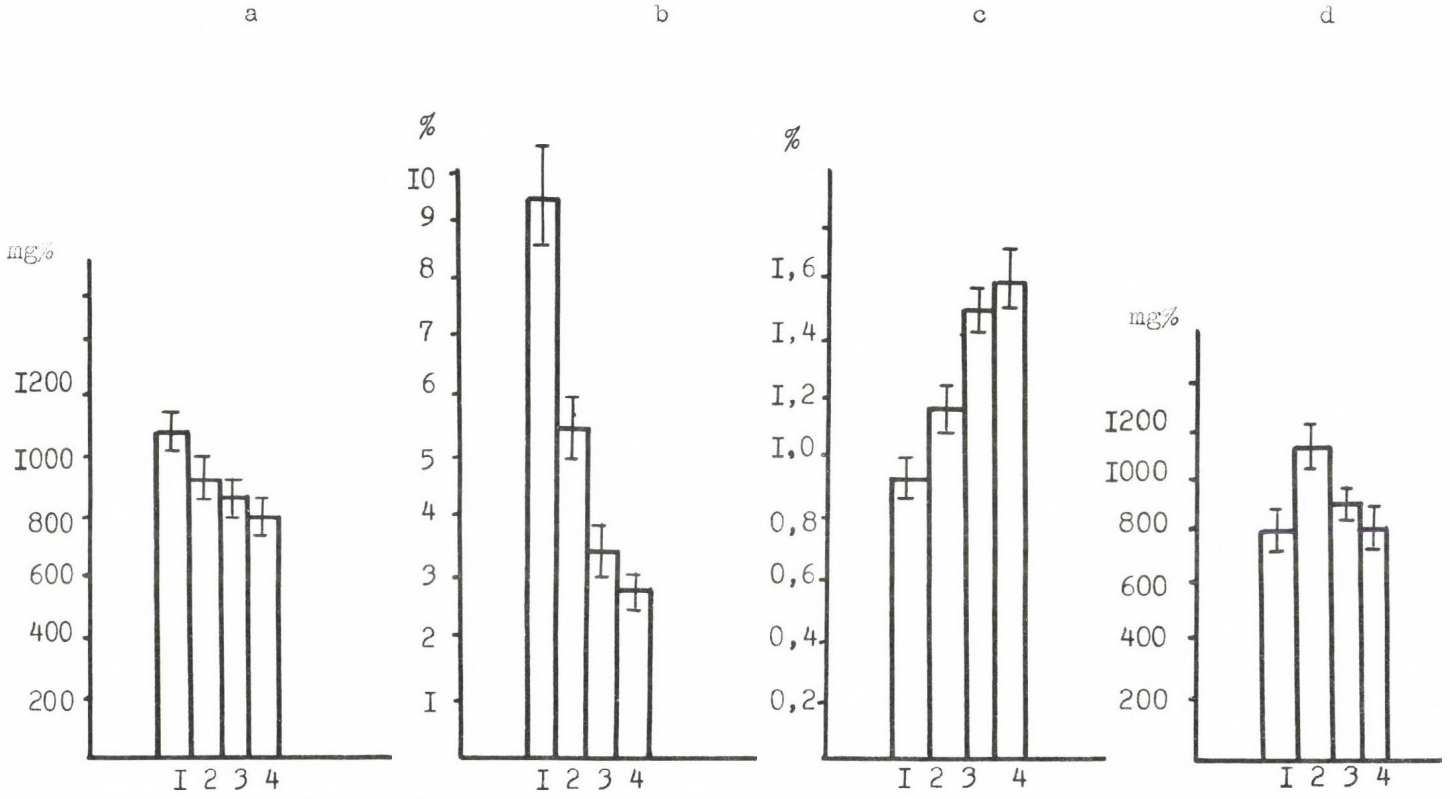

Fig.5 The general lipid content in organs and tissues of a carp depending on the concentration of copper in water: a-blood; b-liver; c-white skeletal muscles; d-bile. 1-0.01 mg/l Cu/control/; 2-0.02 mg/l Cu; 3-0.1 mg/l Cu; 4-1.0 mg/l Cu

tissues of fishes is determined by the concentration of this metal in the water and the duration of its impact on the organism.

Cobalt, like copper, in concentrations exceeding the background ones by 0.01; 0.1; 1.0, and 5.0 mg/l produced different results at different stages of the 7-day adaptation as to the effectiveness and directness of its biological impact upon the biosythesis in the organism of fishes. On the initial adaptation stages of carps /24 h/ we observed the disruption of protein-synthesis function of the liver accompanied by the domination of protein decomposition over their biosynthesis /Fig.6/. However, the adaptation of fishes to the cobalt load brought about not only the restoration of the protein-synthesizing function of the liver but the activating impact of metal on the biosynthesis in the organism as well. There was an inverse relationship between the restoration rate of the liver functions in fishes subjected to the impact of cobalt ions and the cobalt concentration in water.

During the 7-day exposure different concentrations of cobalt in the water activated the consumption of the carboxyl-tagged sodium acetate for the synthesis of lipids. However, the long-term /12 days/ influence of metal on the organism lowered the total of lipids in the liver against the background of their increase in the tissues of the white skeletal muscles and in the bladder bile secreted by the liver which proves the lipotropic impact of this metal.

It should be noted that in contrast to copper, cobalt is less toxic for the biosynthesis in the organism of fishes. There is another evidence of it in the more rapid restoration of biosynthesis in the fish liver under the influence of the boosted metal concentrations in water.

As to the other ions of heavy metals /zinc and manganese/, the obtained data show that up to 1 mg/l excessive background concentrations in the water in model experiments practically did not inhibit the intensity of biosynthesis in the organism. Moreover, these metals in the water mostly activated the biosynthesis of proteins and lipids /Fig.7,8/.

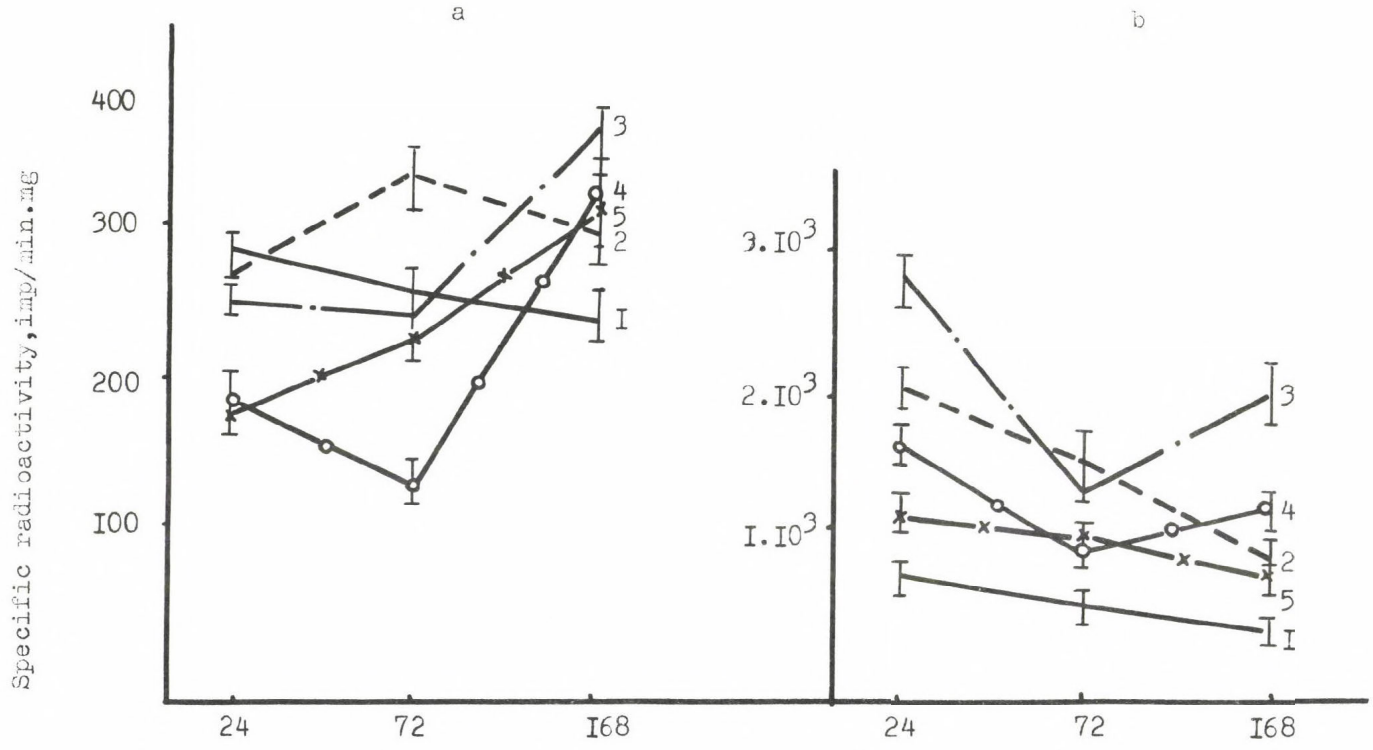

Fig.6 The intensity of incorporation of acetate-2-^{14}C-derived radiocarbon into general albumins /a/ and lipids /b/ in the liver depending on the cobalt concentration in water: 1-0.01 mg/l Co /control/; 2-0.02 mg/l Co; 3-0.1 mg/l Co; 4-1.0 mg/l Co; 5-5.0 mg/l Co

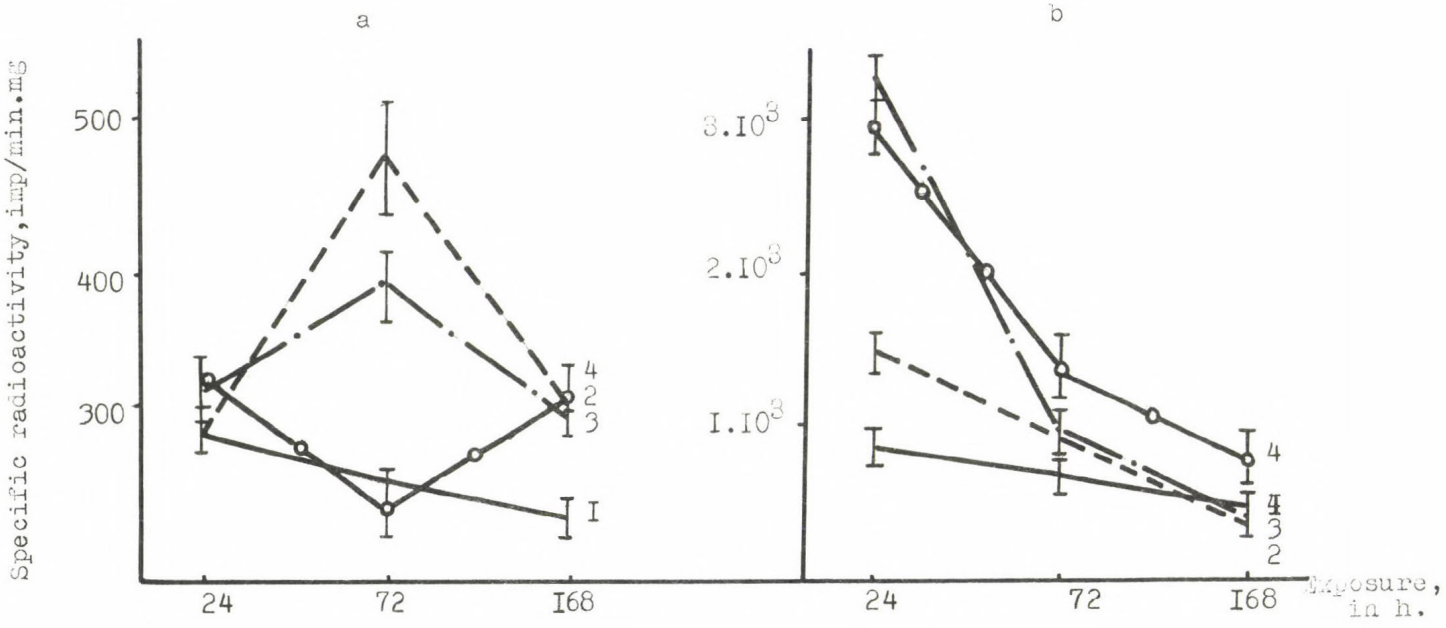

Fig.7 The intensity of tag incorporation from acetate-2-^{14}C into general albumins /a/ and lipids /b/ in fish liver depending on the concentration of manganese in water: 1-0.006 mg/l Mn /control/; 2-0.056 mg/l Mn; 3-0.506 mg/l Mn; 4-1.006 mg/l Mn

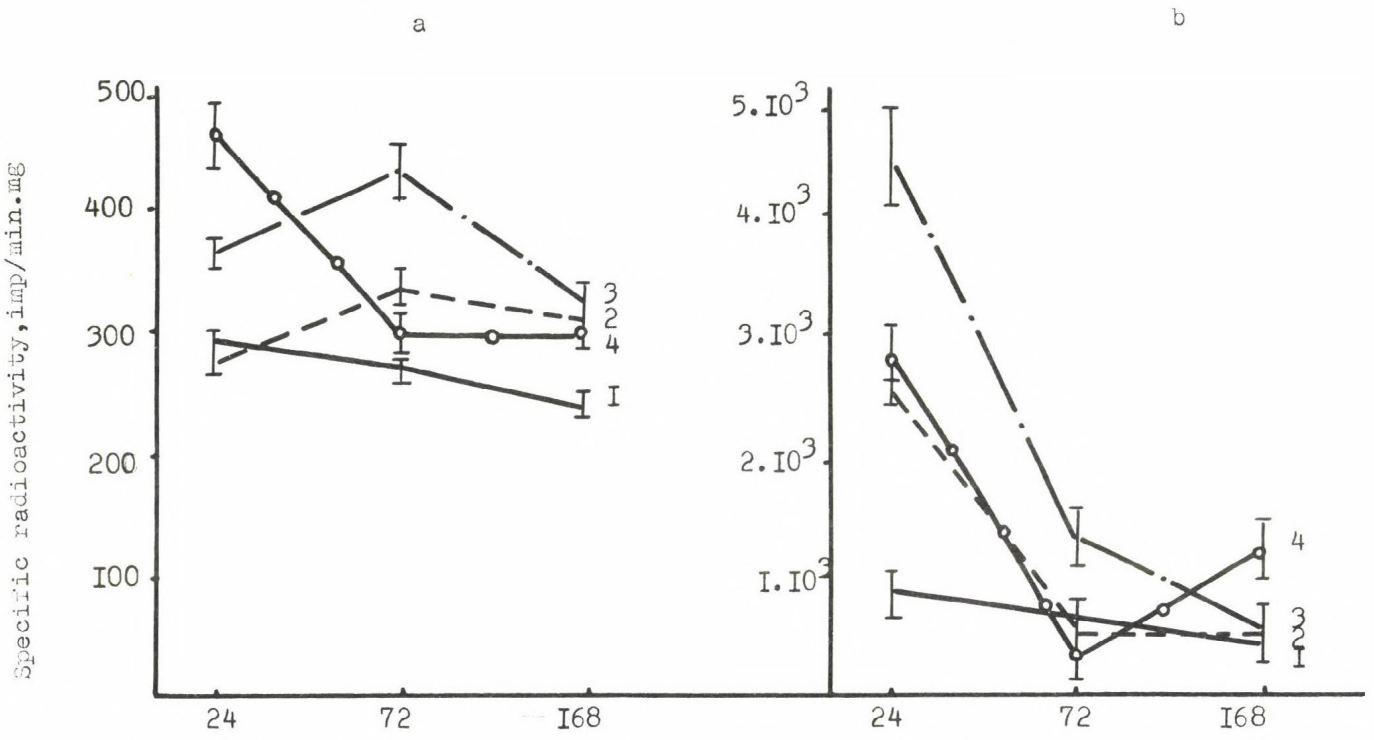

Fig.8 The intensity of incorporation of acetate-2-^{14}C-derived radiocarbon into general albumins /a/ and lipids /b/ in the carp liver depending on the zinc concentration in water: 1-0.003 mg/l Zn /control/; 2-0.013 mg/l Zn; 3-0.103 mg/l Zn; 4-1.003 mg/l Zn

Though there was some difference in the effectiveness of activation in the case of the biosynthesis in the fish organism at various stages of adaptation to the load of microelements depending on the total of the accumulated metal in the organs and tissues. During the experiment, all through the vegetation period /7 days/, we observed fluctuant changes in biosynthesis intensity in the organism caused by the metal content in the organs and tissues of fishes.

So, according to the data of model experiments, both intensity and directness of biosynthesis in the fish organism affected by the heavy metals are determined by their concentration in water, the time of influence, and their accumulation level in the organs and tissues. One should also take into account the physical and chemical properties of the metal itself determining its toxicity toward the hydrobionts.

DISCUSSION

THEEDE, H: You found that the lipid concentration in the muscle tissue could be increased by application of different copper concentrations. Do you have an explanation for this?

JEVTUSHENKO, N.Yu: We think that the coinciding decrease of total lipids in the liver and the increase in the muscles of fish under the effect of various concentrations of copper can be explained by the involvement of copper in the transport mechanisms of lipids from the liver into the lipid depot. One type of them is the white skeletal muscle.

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EXPERIMENTAL STUDY OF BIOACCUMULATION, TOXICITY AND REGULATION OF SOME TRACE METALS IN VARIOUS ESTUARINE AND COASTAL ORGANISMS

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INTRODUCTION

There are a great deal of data on metal concentrations in various estuarine organisms exposed to experimental contamination or taken from areas both free from or subjected to anthropogenic inputs of metals (Jørgensen, 1979 ; Lewis and Cave, 1979 ; Phillips, 1980 ; Fisler, 1981). Parallel with this, numerous scientists taking solely metal concentrations in water into account have determined sub-lethal or acute toxicological parameters (Lewis and Cave, 1979 ; Taylor, 1981 a, b, c). Consequently, the published data do not permit the establishment of direct relations between bioaccumulation and toxicity of metals.

This is all the more regrettable since the concentrations in living organisms are not related to the concentrations in water by a simple invariable function which takes no account of the metal or species. When variations of the chemical composition of the external medium occur, numerous animals are able to keep their internal chemical composition at a steady level, compatible with the normal development of their physiological functions. This ability varies from species to species and generally speaking, the best regulators are the more highly evolved forms, including fish and decapod crustaceans. However, the ability to regulate is effective only for moderate variations of concentrations in the environment. Moreover, this ability seems to vary according to the physiological functions of the trace elements, (Bryan, 1979, 1984 ; Amiard-Triquet and Amiard, 1980).

In consequence, the aim of the present study is to establish the relations between the bioaccumulation and toxicity of essential (Cu, Zn) and non-essential (Cd, Pb) trace metals in estuarine organisms belonging to four zoological groups : annelids (*Nereis diversicolor*), molluscs (*Scrobicularia plana*), crustaceans (*Crangon crangon*) and fish (*Platichthys flesus*).

MATERIAL AND METHODS

The animals were taken from the Loire estuary (*C. crangon* and *P. flesus*) and the nearby coastal area (*N. diversicolor* and *S. plana*). They were then acclimatized to laboratory conditions for a few days. For each species, we selected specimens of similar size and all the experiments were carried out during the same season (winter) so as to

limit the variations due to different physiological states. They were arranged in groups of variable numbers in the experimental containers according to their size and their aggressiveness with regard to their congeners. Ten to fifteen specimens were exposed to each metal concentration for 96 hours.

The breeding was achieved using artificial seawater (25 g of hW Wimex salts per liter of desionized water) which contained 1,68 µg/l of Cd ; 1,58 of Pb ; 2,21 of Cu and 0,59 of Zn. The pollutants were introduced into the breeding medium in the form of chloride for Cd, Cu and Zn and nitrate for Pb. The levels of the experimental additional concentrations of metals are shown in table 1.

Table 1 - Range of experimental inputs of metals.

Species	Metal	Additional concentrations (mg/l)			
Nereis diversicolor	Cd	0,05	0,5	5	50
and *Scrobicularia*	Pb, Zn	0,1	1	10	100
plana	Cu	0,01	0,1	1	10
Crangon crangon and	Cd, Cu, Zn	0,02	0,2	2	20
Platichthys flesus	Pb	0,1	1	10	100

The experimental medium was renewed every day with a view to avoiding or limiting the decrease of metal concentrations in water which is harmful to a good determination of toxicological parameters (Hennig and Greenwood, 1981). In these conditions, no variation of Cd and Zn concentrations occurred. For Cu, a loss of 35 % per 24 h was observed for the highest additional concentration (10 mg/l). For Pb, the decrease per 24 h ranged between - 9 % for the lowest additional concentration (0,1 mg/l) and - 60 % for the highest (100 mg/l).

The experiments were carried out in an air-conditionned room ($15 \pm 0,5$°C) with a nychthemeral rhythm of 12 h/12 h. The animals were starved during the 96 h of exposure.

Dead animals were numbered and removed every day. At the end of the experiments, the surviving specimens were prepared so as to analyse the trace elements. The Cd or Zn contaminated flounders were dissected in order to separate the organs in which these metals are mainly stored (Métayer et al., 1982) namely digestive tract, liver and kidney for Cd; liver, kidney, gills and skin for Zn.

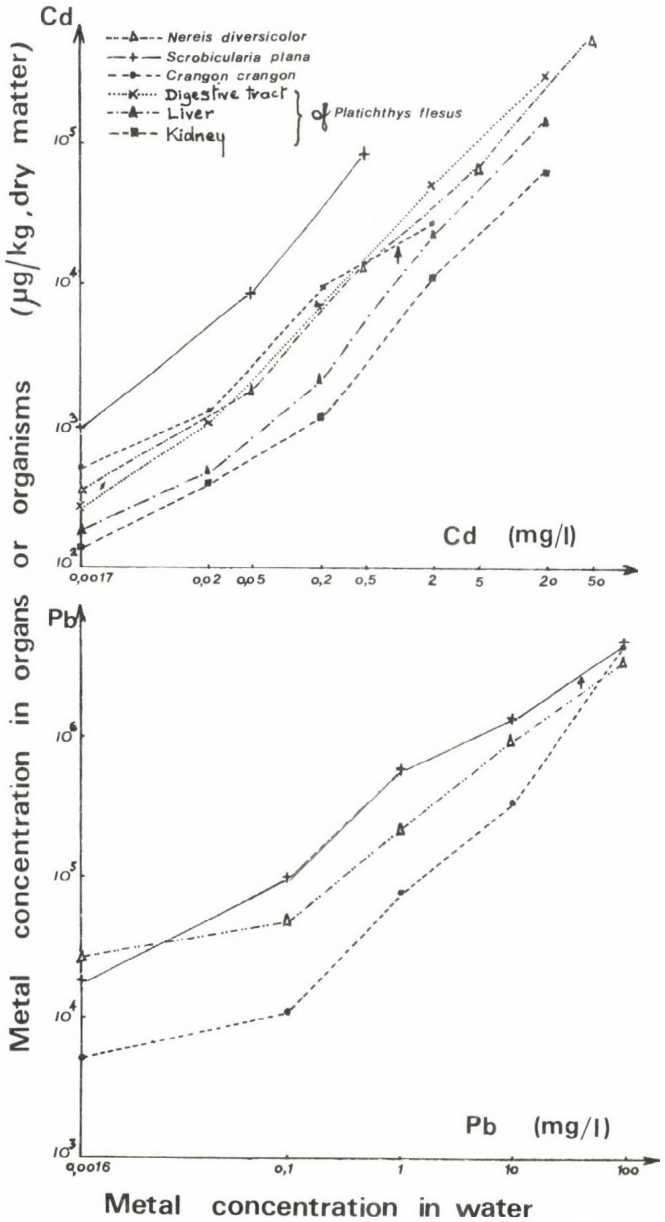
For each experimental concentration, specimens or organs were pooled, oven-dried (80°C) to constant weight and pulverized. For each sample, the trace element analysis was performed in three aliquots of 50 mg of dry matter. After mineralization and extraction using a chloroformic solution of dithizone, metal concentrations were determined by flame (Zn) or flameless (Cd, Pb, Cu) atomic absorption spectrophotometry (Boiteau and Métayer, 1978 ; Amiard-Triquet et al., 1980).

RESULTS

Determination of LC_{50}/96 h

The toxicity of the four studied metals was quantified using a standard toxicological parameter, the lethal concentration 50 % per 96 h

Fig. 1 - Experimental contamination of four estuarine organisms by cadmium and lead.



(LC_{50}/96 h), determined by the statistical technique of Bliss (1938). Results are shown in table 2.

Influence of experimental contaminations on bioaccumulation of metals.

The variations of metal concentrations in organisms related to variations of experimental concentrations in water are represented in figs 1 to 3. In order to make them clearer, only the mean concentrations are shown. The variation coefficients are on average 6 % for Cd and Zn and 8 % for Pb and Cu.

Table 2 - Toxicity of Cd, Pb, Cu and Zn to estuarine organisms

Species	LC_{50}/96 h (mg/l) : Cd	Pb	Cu	Zn
Nereis diversicolor	84*	>100	0,3	49,4
Scrobicularia plana	1,6	43,4	3,2	10
Crangon crangon	0,99	>100	0,8	6,3
Platichthys flesus	> 20	-	-	> 20

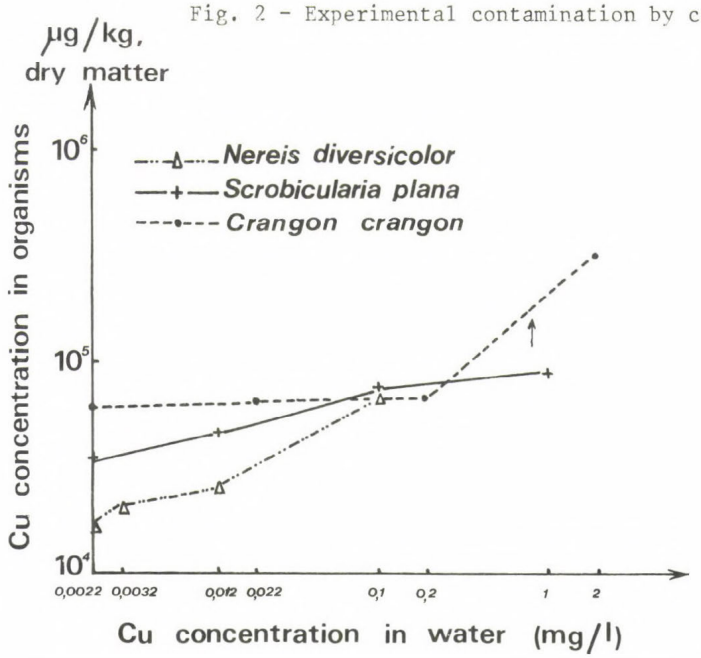
* value obtained by extrapolation.

For all four studied species, the Cd and Pb concentrations in organs or entire organisms increase considerably for ascending amounts of these metals (Fig. 1). The logarithms of these concentrations are linked by a linear function expressed by :

$$\log y = \log a + b . \log x$$

and they are highly correlated (Table 3).

Fig. 2 - Experimental contamination by copper



In the case of copper (Fig. 2), the increase of concentrations in water induces an increase of concentrations in *Nereis* and *Scrobicularia*. The logarithms of these concentrations are significantly correlated but attention must be drawn to the fact that the regression coefficient is lower than for the two previous metals (Table 3). In the shrimp, the copper concentration remains steady for concentrations in water up to 1000 times more than the control concentration and increases only for the highest additional concentration which rises above the LC_{50}/96 h.

Table 3 - Characteristics of the regression relating the logarithms of metal concentrations in organisms to the logarithms of metal concentrations in water (log y = log a + b.log x).

Metal	Species	regression coefficient b	log a	correlation coefficient r*
Cadmium	*Nereis diversicolor*	0,71	4,41	0,99
	Scrobicularia plana	0,78	5,10	0,99
	Crangon crangon	0,61	4,31	0,98
	Platichthys flesus			
	digestive tract	0,78	4,46	0,99
	liver	0,74	4,10	0,98
	kidney	0,68	3,81	0,98
Lead	*N. diversicolor*	0,47	5,46	0,95
	S. plana	0,52	5,66	0,99
	C. crangon	0,61	5,08	0,95
Copper	*N. diversicolor*	0,35	5,16	0,98
	S. plana	0,16	4,99	0,98
	C. crangon	0,22	5,26	0,81
Zinc	*N. diversicolor*	0,09	2,40	0,81
	S. plana	0,04	2,78	0,89
	C. crangon	0,07	1,77	0,99
	P. flesus			
	liver	0,09	2,24	0,88
	kidney	0,04	2,36	0,84
	gills	0,05	2,47	0,76
	skin	- 0,01	2,51	0,30

* r significant at the 1 % level ; r : 5 % ; r : 10 %.

For Zn, the regression coefficients relating logarithms of concentrations in organs or organisms to logarithms of concentration in water are very low. Indeed, for the highest input assayed which is 33000 times higher than the concentration in controls, the zinc concentration in contaminated organisms is at the maximum level 3,2 times greater than the natural one. In order to go into greater detail,

Fig. 3 - Experimental contamination of four estuarine organisms by zinc.

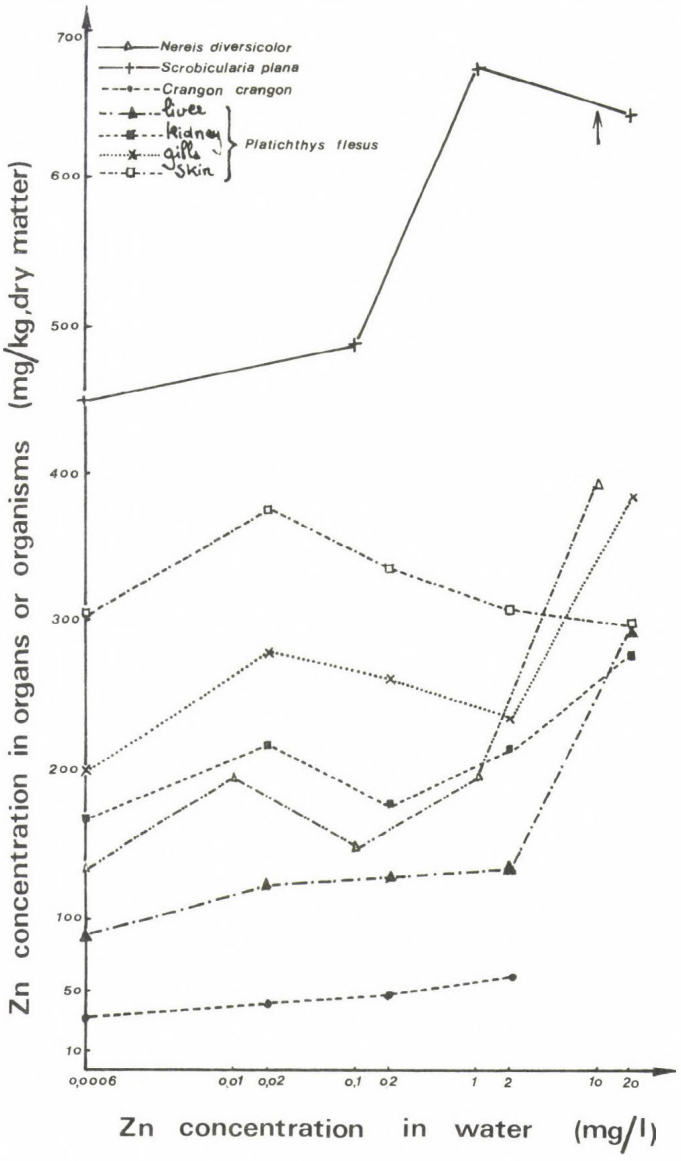


figure 3 has been visualized using semi-logarithmic coordinates which allow an exaggeration of the scale of concentrations in organisms. In *Scrobicularia*, the concentration of Zn increases noticeably, only for additional concentrations in water greater than 0,1 mg/l (that is to say more than 167 times the concentration in controls). For the other three species, the concentrations in water must be even higher to induce an appreciable increase of the bioaccumulation of Zn.

DISCUSSION AND CONCLUSION

For cadmium, the experiments related by Phillips (1980) demonstrate the ability of molluscs, crustaceans and fish to rapidly take up Cd in solution. In flat fish (*Limanda limanda, Pleuronectes platessa*), Westernhagen et al. (1980) have shown a significant accumulation of Cd following experimental contaminations (5 and 50 µg per l). In *Crangon crangon*, the experimental contamination of water by cadmium (1,5 to 100 µg/l) induces a fast uptake of this metal leading to a temporary state of equilibrium. This is followed by a long term accumulation, characterized by a linear increase of the whole quantity of Cd in the organism (Dethlefsen, 1977-78). In *Nereis diversicolor* and *Scrobicularia plana* from more or less polluted environments, the level of cadmium in the organisms and that in the sediment vary in a similar way (Bryan et al., 1980 ; Amiard et al., 1982).

In situ studies of the bioaccumulation of lead have shown that the levels of this metal in *N. diversicolor, S. plana* and *M. balthica* depend on the contamination of the sampling site, estimated from lead concentration in sediments (Bryan et al., 1980 ; Amiard et al., 1982). In *Crangon crangon* and in the freshwater crustacean *Orconectes virilis*, the concentrations of lead in the organisms depend on the level of exposure to this metal in the environment (Anderson and Brower, 1978 ; Amiard-Triquet et al., 1983). The same phenomenon is observed in the flat fish *Solea solea* and *Platichthys flesus* (Amiard-Triquet et al., 1983). In trout exposed to 3 to 120 µg Pb/l, the logarithm of Pb concentrations in most of the tissues varies linearly according to the logarithm of concentrations in seawater (Hodson et al., 1978).

In situ, Cu concentrations in *Nereis* follow the variations of Cu concentrations in sediment whereas the level of Cu in *Scrobicularia* seems comparatively independent (Bryan et al., 1980 ; Amiard et al., 1982). White and Rainbow (1982) have shown the ability of the crustacean *Palaemon elegans* to control the level of copper in its tissues for external concentrations as high as 100 µg/l. For *Crangon crangon*, the present study indicates that the threshold beyond which the internal Cu is no longer regulated is higher than 200 µg/l. The levels of copper in *P. elegans* and *C. crangon* are similar regardless of the sampling areas. This is in agreement with the experimental data indicating a biological regulation of Cu in these species.

For Zn, we do not observe any differences between *N. diversicolor* and *S. plana*, sampled from two areas which differ in their level of contamination such as it can be evaluated according to Zn concentrations in sediment (Amiard et al., 1982). This corresponds with the experimental data of the present study which indicate that Zn concentrations in organisms are virtually independent of external levels for inputs lower or equal to 100 µg Zn/l in *S. plana* and 1 mg Zn/l in *N. diversicolor*. Bryan et al. (1980) detect concentrations as high as 466 mg/kg (dry matter) in *N. diversicolor* and 4920 in *S. plana* sampled from heavily polluted British estuaries. These concentrations are higher than those which have been reached in organisms exposed to experimental concentrations nearing LC_{50}/96 h. This apparent discrepancy could be explained by the fact the organisms may have acquired a tolerance towards pollutants such as those which have been described for *Nereis diversicolor* (Bryan and Hummerstone, 1973).

The ability of crustaceans to regulate their Zn concentration in environments where the level of this metal is variable has been

established experimentally for several species (Bryan, 1966 ; Ray et al., 1980 ; White and Rainbow, 1982). *In situ* studies show that Zn concentrations in the shrimps *C. crangon* and *Palaemon elegans* are widely independent of the environmental level of this metal (White and Rainbow, 1982 ; Amiard-Triquet et al., 1983). The results of the present study confirm that the uptake of Zn by *C. crangon* is only fractionally disturbed for additional concentrations as high as 2 mg/l.

For fish, there are very little published data available. In the fresh-water species *Scardinius erythrophtalmus*, Van Hoof and Van San (1981) have shown that, for a lethal concentration reaching 42 times the concentration in controls, the kidney is the only organ which exhibits a significant Zn uptake. No significant increase can be observed in muscles, gills, gill cover or liver. The same holds true for animals coming from a polluted area. In the flounder, the present study has shown that additional concentrations higher than 2 mg Zn/l are necessary to induce a limited increase of Zn concentrations in gills, kidneys and liver whereas inputs as high as 20 mg/l do not disturb the level of Zn in skin. *In situ*, no difference may be observed between estuarine and coastal flounders gathered from areas characterized by different levels of Zn in sediments (Amiard-Triquet et al., 1983).

The experimental and *in situ* data of the literature and of the present study clearly show that the increase of Cd and Pb concentrations in the external medium induces an increase of the level of these metals in the organisms. For Zn, all the studied species can maintain their internal concentration at a steady level for a large range of external concentrations and, even for inputs approaching lethal concentrations (LC_{50}/96 h). For Cu, the regulation differs according to the zoological group. It is mainly effective in crustaceans in which Cu is an essential trace metal as a constituent of the oxygen carrier, haemocyanin. We therefore agree with the theory of Bryan (1979, 1984) who contrasts essential metals (Zn, Cu, Mn...), which can be regulated by living organisms, at least in some measure, with noxious metals (Cd, Pb...) the internal level of which depends on their external level.

Table 4 - Comparative tolerance of organisms to the presence of trace metal in their tissues (Ratios between metal concentrations in the organisms for experimental inputs immediatly below LC_{50}/96 h and concentrations in controls)

Species	Cd	Pb	Cu	Zn
Nereis diversicolor	1428	130	4	2,9
Scrobicularia plana	91	79	2,6	1,5
Crangon crangon	20	900	1,1	1,8
Platichthys flesus				
Liver	807	-	-	3,2
Kidney	485	-	-	1,7
Digestive tract	1200	-	-	-
Skin	-	-	-	1,0
Gills	-	-	-	1,9

However, attention must be drawn to the fact that the non-essential toxic metals Cd and Pb can be tolerated in concentrations far beyond normal tissue levels whereas essential metals Zn and Cu become lethal for concentrations scarcely higher than normal ones. Table 4 shows the ratios between metal concentrations in the organisms corresponding to the

experimental input immediatly below LC_{50}/96 h and concentrations in controls. For Cd an Pb, this ratio varies from some tens to more than 1000 whereas for Cu and Zn, it reaches 4 at the maximum. However, the toxicity of a metal does not depend only on its concentration but also on its physico-chemical form in the organism. So it does not seem unlikely that at least part of Cd and Pb may be stored as harmless forms such as metallothionein, insoluble salts stored in special cells, renal or hepatopancreatic concretions.

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DISCUSSION

SOVOBODOVÁ, Z: You were measuring the lethal concentrations. Do you have a collection of standard methods of toxicological tests concerning water organisms? For which organisms? Do you also have a standard method for the testing of toxicity using fishes and which species of fishes do you use?

AMIARD-TRIQUET, C: We try to prepare new sub-lethal tests able to show the effect of low levels of pollutants. Therefore, we study the disturbance of certain behaviours /phototropism of crustacean larvae/ or of certain biochemical mechanisms /amino acids, enzymes and hormones/ mainly in crabs.

THEEDE, H: Your experiments were carried out with unfed animals. Do you think that feeding may have an influence on the regulating capacity with respect to heavy metals?

AMIARD-TRIQUET, C: We may imagine that starvation could disturb the biological mechanisms of regulation but in short-term experiments like those reported here, the phenomenon, if it exists, is probably not important. Moreover, the results of field studies, in which animals are fed ad libitum, are very similar to experimental results with starved organisms.

THEEDE, H: I think the reported results are very important because they again stress that trace elements as Cu and Zn, which are released in great amounts to the environment, can be as dangerous or even more than the often mentioned non-essential elements Cd and Pb.

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UPTAKE AND RELEASE OF MERCURY AND CADMIUM IN VARIOUS ORGANS OF MUSSELS (*ANODONTA CYGNEA* L.)

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Accumulation of heavy metals in aquatic organisms - similarly to other living systems - is the result of two opposite processes, namely the net result of uptake and release. In case uptake is faster than release, accumulation takes place, in case the two processes are equal, there is an equilibrium.

The mechanisms which are responsible for the uptake and release may function differently depending on the chemical character of the substance taken up by the organism, and often also the environmental concentration of the substance is of primary importance. At low concentrations equilibration usually takes place at a low level of accumulation, while higher concentrations may lead to high accumulation. The best biomonitors for detection of pollution are the organisms with a great accumulation capacity, the regulatory mechanism of which are week to keep the equilibrium of uptake and release at low level even at low environmental contamination.

It is known that mussels are good accumulators for a number of heavy metals (Pringle et al. 1968). Since they are characterized by a filter feeding behvior, when they pump the water through the gills and take up large amounts of small particles as food, they ingest various substances dissolved in the water or incorporated into algae, bacteria and other particulate substances.

In our earlier investigations we showed that the fresh water mussel Anodonta cygnea L., living in Lake Balaton, similar to the other molluscan species, accumulates high amounts of

heavy metals, and its concentration capacity may reach a value of several ten thousands in various organs as compared to the concentration of the metal in the surrounding water (Salánki et al. 1982). The concentration capacity of various organs is different, the highest amount of accumulated metals was detected in the kidney and gills, while the lowest levels were detected in the adductors (V.-Balogh and Salánki 1984).

The great concentration capacity of mussels to heavy metals allows us to use these animals in biomonitoring the heavy metal pollution of the environment which may occur as a result of various kinds of human activities. The question arises however, whether temporary changes of heavy metal concentrations in the environment will be reflected in the heavy metal content of mussels or not, and, on the other hand, whether changes in the heavy metal content of mussels reflected changes in the environment or it may be connected with mechanisms within the animal regulating uptake and release of these substances.

In the literature there are contradictions whether heavy metals will be released by mussels, or not, in case the surrounding metal concentration drops. Mason et al. (1976) did not found release of Hg from oysters within 256 hours. The same was reported for Pb and Cd by Klumpp and Burden-Jones (1982) in Trichomya hirsuta. On the other hand, Cunningham and Tripp (1973) found a considerable release of accumulated Hg by oysters kept in Hg-free water. Fowler et al. (1978) described also release of Hg by Mytilus, however, to a much lesser degree. They also found a redistribution of the metal within the animal during the depuration period. Looking for the release of Cd Zaroogian (1979) did not find real depuration within 16 weeks from oysters having been previously kept in a 15 µg/l solution of cadmium.

In a series of investigations we translocated mussels (Unio sp.) of a low metal content from Lake Balaton into the River Zala which is the main inflow into the lake. Starting at early spring, we took samples from these mussels at two-week intervals for measuring heavy metal concentration in the gills (V.-Balogh and Salánki 1983). For Hg, Cd, Pb and Zn continual increase was found, however, on some occasions, also a considerable drop in

metal concentrations. These results suggest that the metals taken up by mussels are released if the surrounding medium changes, and so mussels can be used as biomonitors for detecting fluctuation of heavy metal pollution. Nevertheless, it seems necessary to carry out laboratory experiments and to clarify the dynamics of uptake and release of toxic metals in vaious organs of the mussels. We report here our results concerning uptake and release of Hg and Cd in the tissues of Anodonta cygnea L.

MATERIAL AND METHODS

In the experiments 11.9±1.01 cm long specimens of Anodonta cygnea were used taken from fish ponds. The animals had been kept in running Balaton water for weeks before the heavy metals were added. No additional feeding was performed. Two separate series of experiments were carried out both with Hg and Cd. First we investigated the uptake of metals both at short intervals during 24 hours and at long intervals during up to 840 hours. In the second series after an 840 hours' uptake we studied depuration, in case of Hg for 840, in case of Cd for 672 hours. Hg was applied in the form of $HgCl_2$, while Cd as $CdSO_4$. The following system during the uptake experiments ensured a constant metal concentration which was controlled in the outflow from time to time.

When investigating only uptake the animals were kept separately in 3 l volume Plexyglass chambers. This amount of water was renewed within about one hour due to the perfusion system. The concentration of Hg and Cd were 10±7 and 16±5 µg/l, respectively. Temperature of the water changed between 7-15 °C.

In the second series of experiments when long-term uptake and release were investigated, 100 animals were placed together in a large wessel containing 100 l water. The in- and outflow assured the total change of the water within 5 hours. The Hg and Cd concentrations were 12.9±2.8 and 6±1.3 µg/l, respectively. The water temperature varied between 15-22 °C.

For measuring heavy metal concentrations in each case 3 animals were used parallelly. Gills, adductor muscles, mantle,

kidney and foot were sampled separately. In case of measuring Hg the whole foot (including viscera) was used, while in Cd measurements only of the viscera located in the foot were analysed.

Tissue samples for Hg were prepared according to Paus (1972), and for Cd measurements according to Krishnamurty et al. (1976) Measurements were carried out by atomic absorption spectrophotometry as it was published earlier (Salánki et al. 1982)

The concentration factor was calculated according to Taylor (1983), $CF=\frac{Ce-Cc}{Cs}$, where Ce=metal concentration in the organ at the end of the exposure, Cc=metal concentration in the organ of the control animal, Cs=metal concentration of the water during the experiment.

RESULTS AND DISCUSSION

Concentrations of Hg and Cd in the organs of Anodonta cygnea L. before exposure to heavy metals are given in Table 1.

Table 1: Concentration of Hg and Cd in control animals (µg/g dry w±SEM)

Organ	Hg		Cd	
	1st series	2nd series	1st series	2nd series
gills	1.21±0.931	0.669±	3.49±0.383	4.24±0.824
foot	1.19±0.786	0.201±0.123	--	--
adductors	1.33±0.806	0.868±0.577	3.67±1.03	4.94±1.29
mantle	1.25±0.088	0.397±0.155	2.67±0.556	3.89±1.88
kidney	1.74±0.692	1.051±	11.6 ±0.961	10.3 ±1.00
viscera	--	--	4.45±0.416	6.36±0.670

The values show that there were some differences between the control concentrations in the two series of experiments, especially for mercury. Nevertheless, due to the large uptake of metals during exposure, we do not ascribe importance to these differences when evaluating the dynamism of uptake and release.

UPTAKE AT THE BEGINNING OF EXPOSURE

Following exposure of mussels to 10 µg/l Hg solution the concentration of Hg increased in all organs within 30-60 min, however, in a few hours it dropped everywhere below the control (Fig.1). Following further exposure an increase started again first in the gills and later in all organs, reaching a moderate elevation by 24 hours. The concentration of Cd increased during the first 24 hours following the exposure of mussels to 16 µg/l Cd only in the kidney, which was noticeable already within 1 hour (Fig.2), but it also decreased somewhat between 12 and 24 hours.

UPTAKE OF Hg AND Cd DURING 840 HOURS' EXPOSURE

Taking samples after 72, 168, 336, 504, 672 and 840 hours' exposure we found that the uptake of both Hg and Cd increased linearly in most organs during this period (Table 2). Our results are in agreement with the findings of Mason et al. (1976) who described in oysters a two-phase uptake, the first phase being logarithmic, while the second one linear. Nevertheless, in gills a saturation with Hg and in the kidney and viscera with Cd were found, since after 672 hours of exposure to 10, and 16 µg/l mercury and cadmium concentrations, respectively, no further increase of the metal concentration was observed.

At the end of the exposure period the concentration factor (CF) was rather different for different organs (Table 3). Accumulation of mercury was of a higher degree than that of the cadmium, and there were also differences between the two metals according to the affinity of organs. The kidney revealed very high CF for mercury, while the viscera and mantle showed prominent values for Cd. The lowest accumulation was observed for both metals in the adductor muscles.

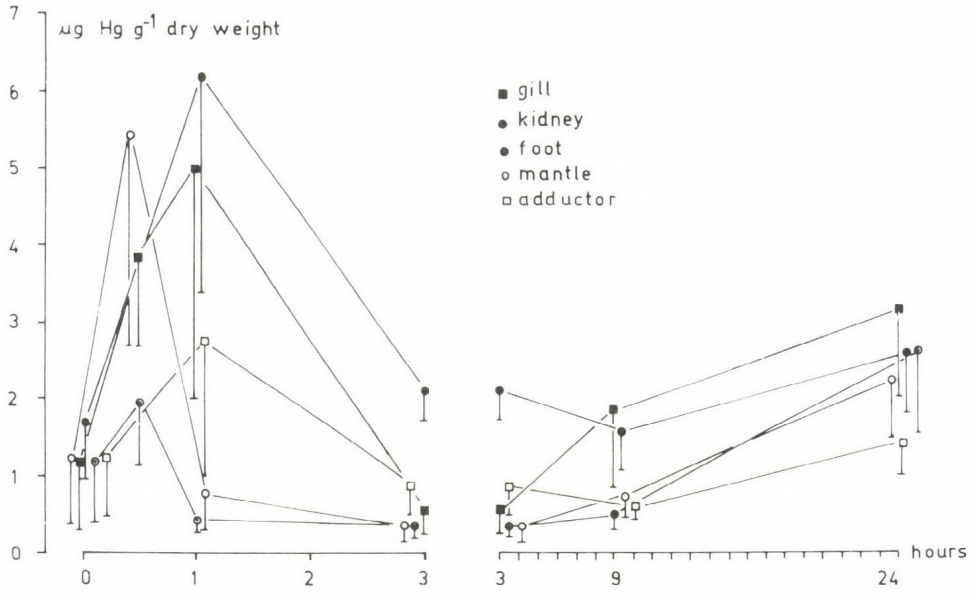

Fig.1. Uptake of mercury into various organs of Anodonta cygnea L. exposed to 10 µg/l solution of Hg during the first 24 hours

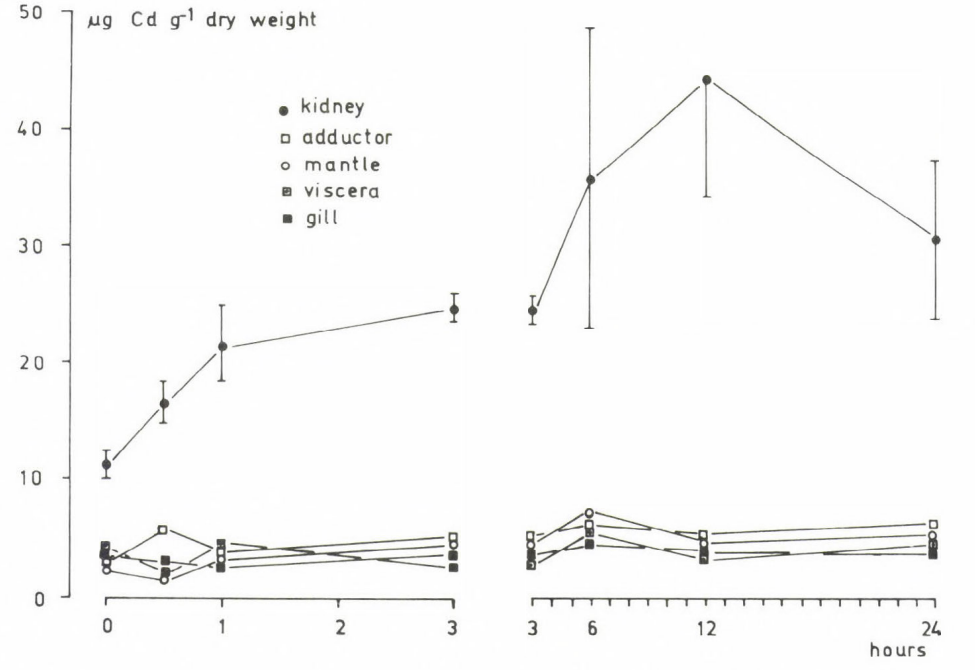

Fig. 2. Uptake of cadmium into various organs of Anodonta cygnea L. exposed to 16 µg/l Cd during the first 24 hours

Table 2. Linear uptake of Hg and Cd in organs of Anodonta cygnea L. during 72-840 hours' exposure

Organ	Hg /10 μg l^{-1}/	Cd /16 μg l^{-1}/
gills	⊗	y = 3.61 + 0.028 x r = 0.782
foot	y = -2.79 + 0.074 x r = 0.851	-
adductors	y = 0.462 + 0.031 x r = 0.846	y = 6.43 + 0.010 x r = 0.593
mantle	y = -6.46 + 0.136 x r = 0.883	y = 2.49 + 0.057 x r = 0.945
kidney	y = -28.6 + 0.593 x r = 0.826	⊗
viscera	-	⊗

⊗ Saturated before 840 hours
y = equation of the line
r = regression coefficient

Table 3. Concentration factor (CF) in various organs of Anodonta cygnea L. after 840 hours' exposure

Organ	Hg /10 μg l^{-1}/	Cd /16 μg l^{-1}
gills	8000	2000
foot	7000	-
adductor muscle	3000	500
mantle	10000	3000
kidney	50000	2000
viscera	-	3000

DEPURATION OF Hg AND Cd

In this second series of experiments with mercury lasting altogether for 1680 hours and with cadmium for 1512 hours during the uptake period we measured the metal concentrations of the organs three times, namely at 168, 504 and 840 hours of the exposure. Following 840 hours' exposure, the animals were washed in running, metal-free Balaton water. Samples were taken at

72, 168, 336, 504, 672 and 840 hours to check mercury depuration, while for measuring cadmium depuration the last sample was taken at 672 hours. We could not sample mussels treated with cadmium at 840 hours of depuration, because the mortality of cadmium-treated animals became very high after placing them into metal-free water.

Elimination of mercury

Mercury, taken up within 840 hours was not released in equal degrees from various organs, and there was no total depuration from either of them during the experimental period. Depuration of mercury was fastest from the kidney (Fig.3), the adductor muscle (Fig.4) and the mantle (Fig.5), where decrease of the accumulated metal to 50 per cent was observed between 72 and 168 hours ($T_{1/2}$=half depuration time). Nevertheless, even after 840 hours these organs contained 35, 7 and 54 times more

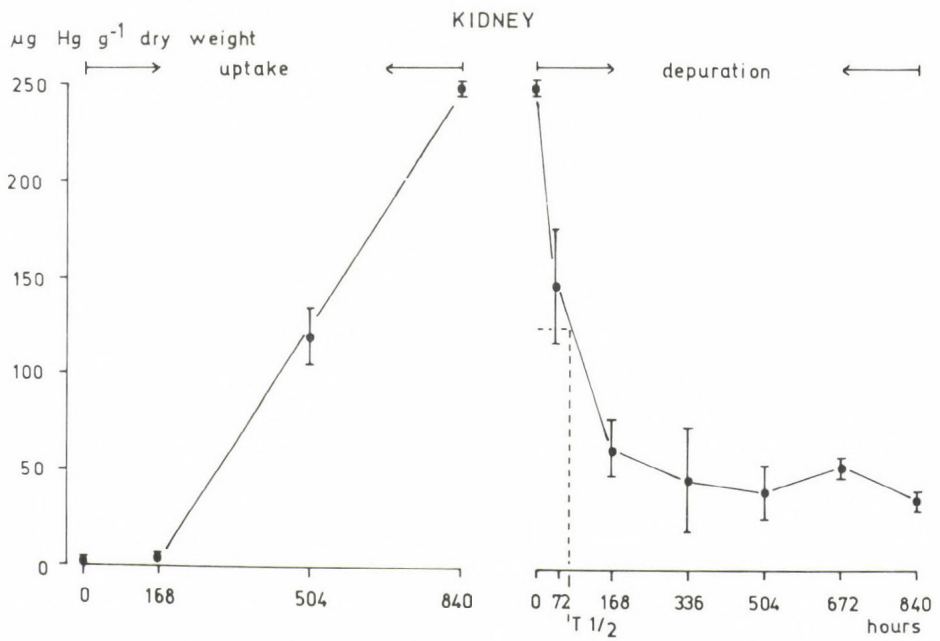

Fig.3. Changes of concentrations of Hg in the kidney of Anodonta cygnea L. during uptake and depuration experiments. $T_{1/2}$=time, necessary for the 50 per cent decrease of the metal concentration.

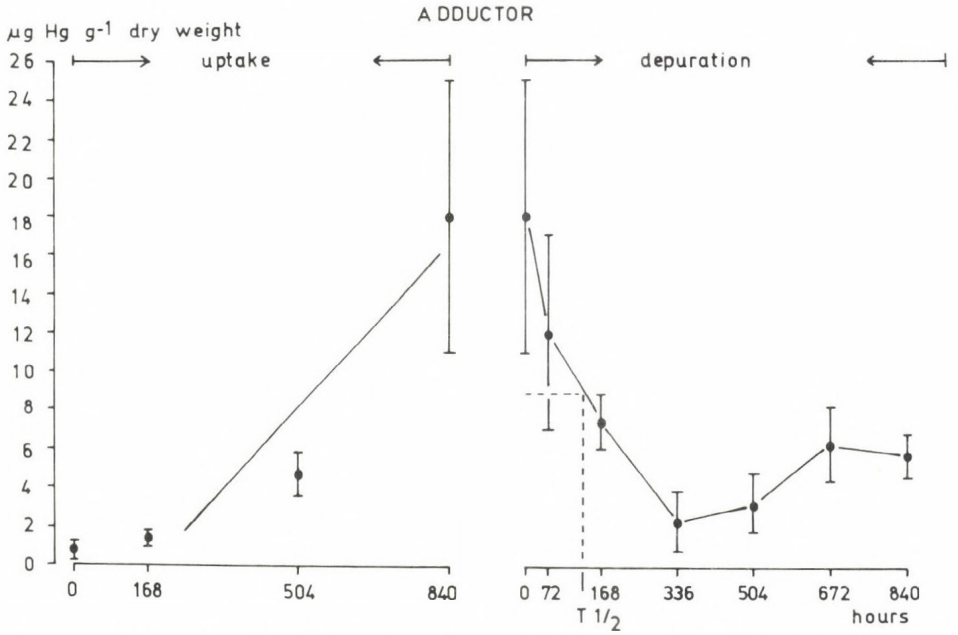

Fig.4. Changes of concentrations of Hg in the adductor muscle of Anodonta cygnea L. during uptake and depuration experiments

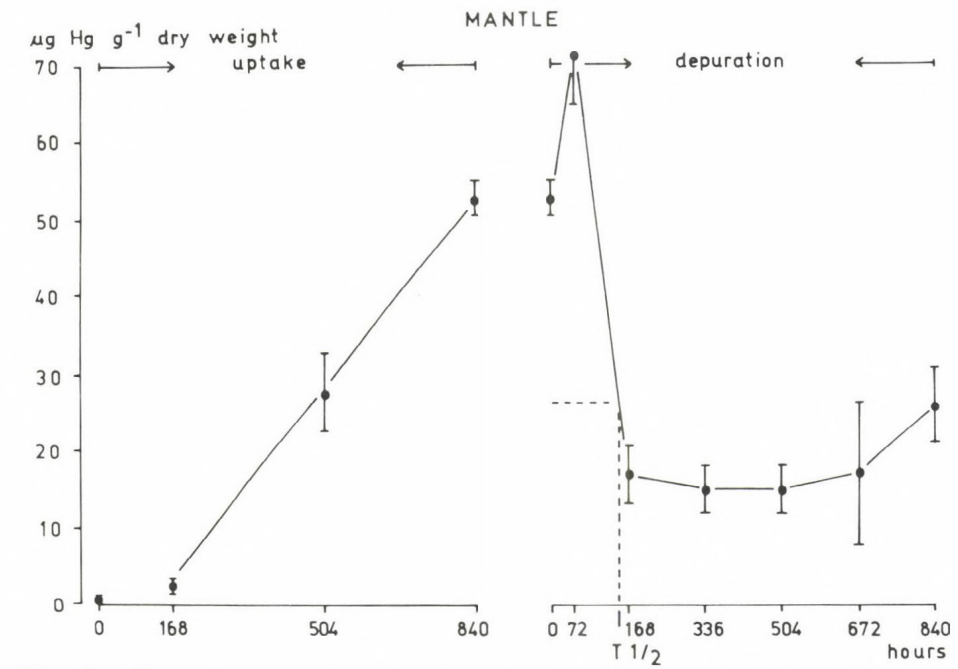

Fig.5. Changes of concentrations of Hg in the mantle of Anodonta cygnea L. during uptake and depuration experiments

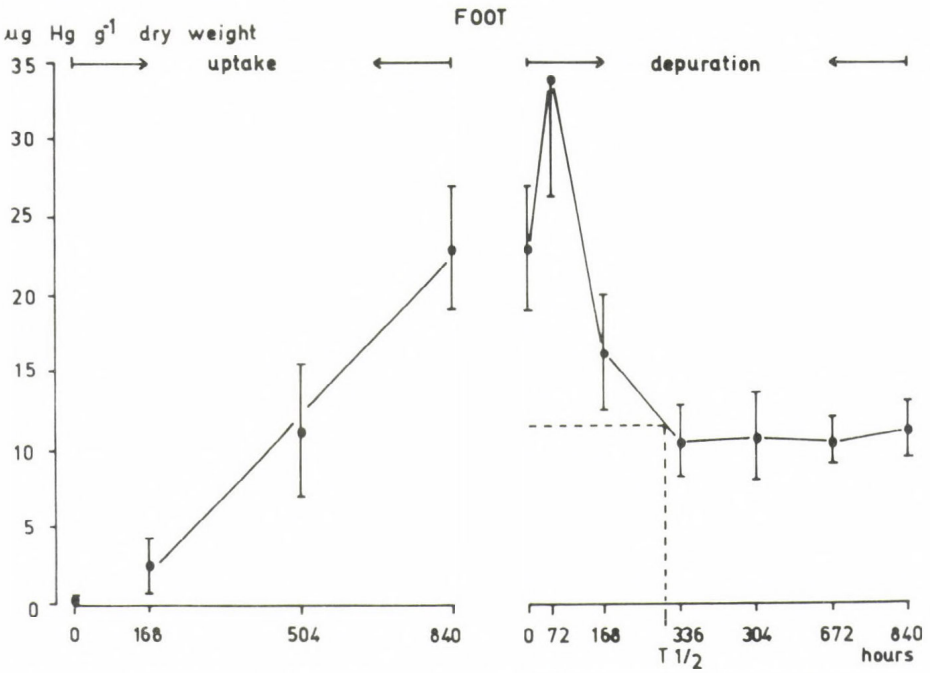

Fig.6. Changes of concentrations of Hg in the foot of Anodonta cygnea L. during uptake and depuration experiments

mercury than the control. $T_{1/2}$ for the foot (Fig.6) was between 168-336 hours, and this level, exceeding the control 54 times, remained constant to the end of the experiment. Less depuration was observed in gills (Fig.7), where $T_{1/2}$ had not been reached during 840 hours, although a definite depuration occurred.

Elimination of cadmium

Binding of cadmium was in each organ stronger than that of mercury. $T_{1/2}$ was between 504 and 672 hours for the mantle (Fig.8) and the gills (Fig.9). There was an obvious depuration from the adductors (Fig.10), however, no half depuration was observed up to 672 hours. In case of the viscera (Fig.11), a 20-25 per cent decrease of cadmium concentration was measured within one week, however, further release was not observed. The kidney (Fig.12) did not release cadmium within 672 hours, there was even a transient increase of cadmium concentration.

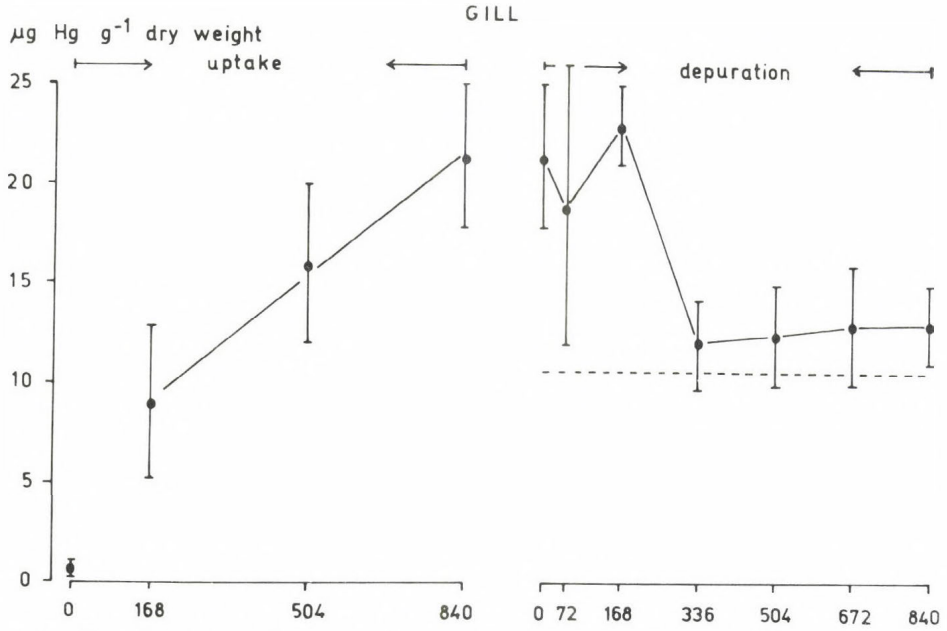

Fig.7 Changes of concentrations of Hg in the gills of Anodonta cygnea L. during uptake and depuration experiments

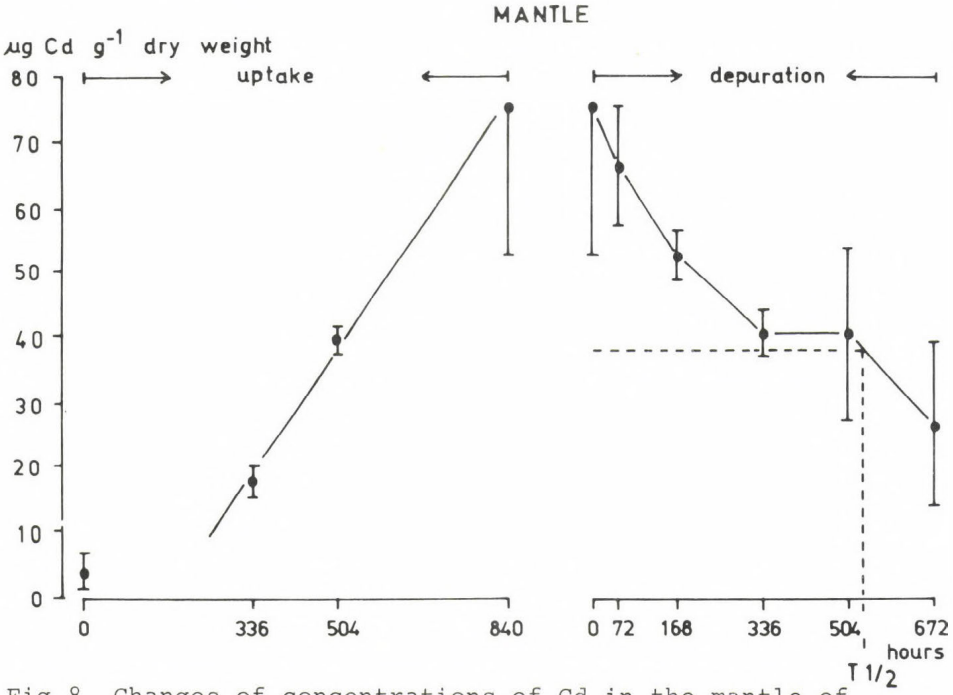

Fig.8 Changes of concentrations of Cd in the mantle of Anodonta cygnea L. during uptake and depuration experiments

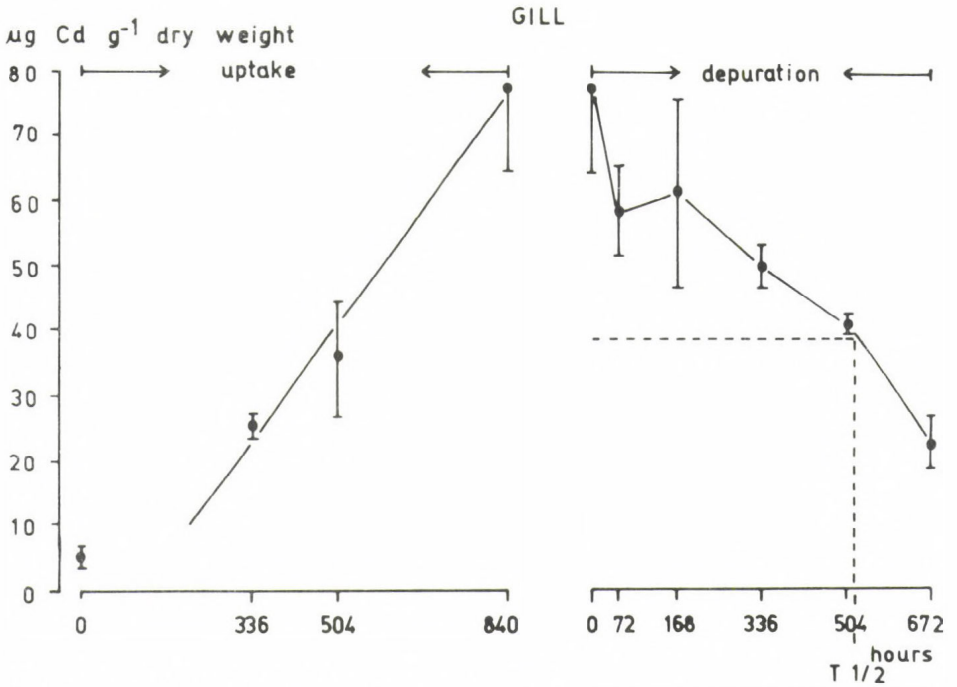

Fig.9 Changes of concentrations of Cd in the gills of Anodonta cygnea L. during uptake and depuration experiments

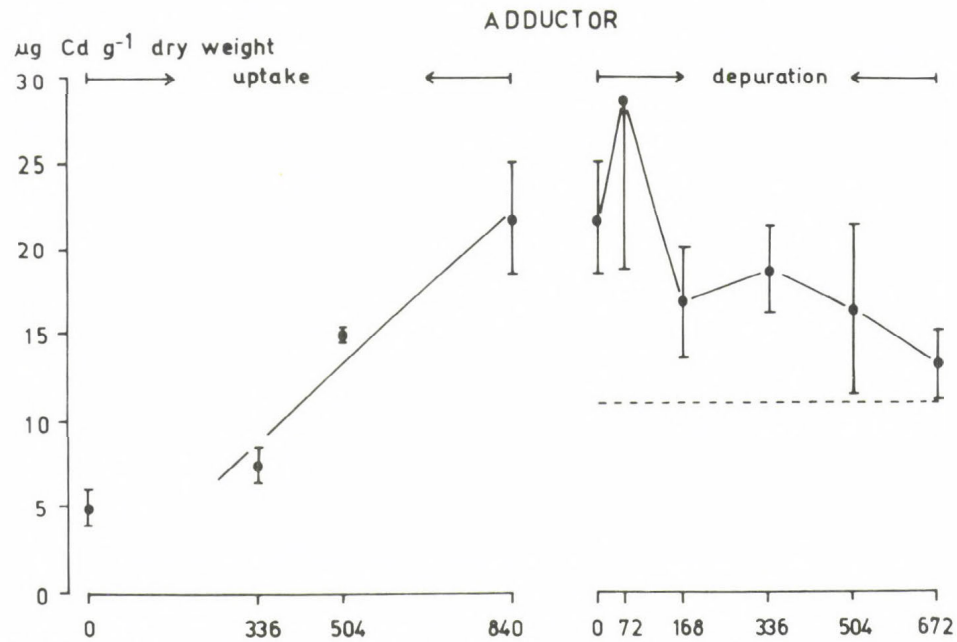

Fig.10 Changes concentrations of Cd in the adductor muscles of Anodonta cygnea L. during uptake and depuration experiments

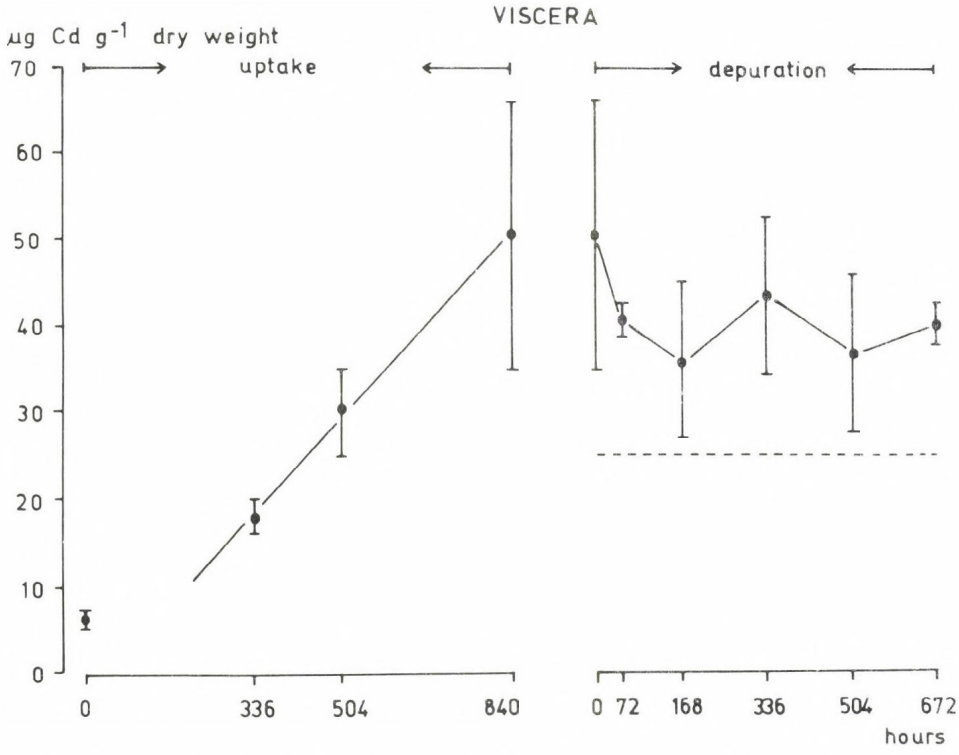

Fig.11 Changes of concentrations of Cd in the viscera of Anodonta cygnea L. during uptake and depuration experiments

Our results are in agreement with the data of Ruzic (1972) Mason et al. (1976), George and Coombs (1977) and others, showing that heavy metal accumulation has two phases in mussels, the first being a reversible one, while the other an irreversible process. In our experiments we found that the various organs accumulate heavy metals to a different degree, the order for mercury being: kidney> gill> mantle> foot (viscera)> adductor muscle, while for cadmium: kidney> viscera> mantle> gill> adductor muscle. The prominent role of the kidney in metal uptake was emphasized also by Bryan (1973) and Carmichael et al. (1980). It is noteworthy that, among the organs, the gill was a better accumulator for mercury than for cadmium. This refers to a difference in the mechanism of uptake and storage of Hg and Cd by the gills, although both metals are known to bind to the SH groups of proteins (Vallee and Ulmer 1972).

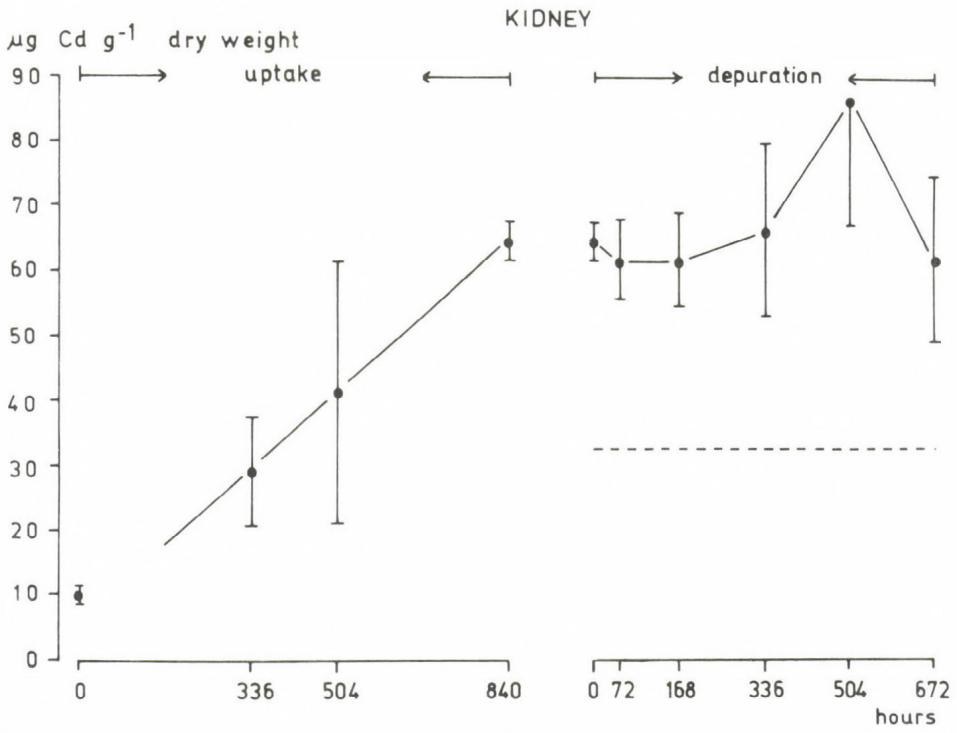

Fig.12 Changes of concentrations of Cd in the kidney of Anodonta cygnea L. during uptake and depuration experiments

Not only uptake but also depuration of Hg and Cd were different from various organs of mussels. In general, release of Hg was faster than that of Cd. The order of organs for the release of Hg was: kidney > adductor muscle > mantle > foot > gills. The gills kept more than half of the Hg after 840 hours' depuration time. Although in similar conditions the accumulation of Cd was less than that of Hg, the release was slower, and the order of organs was mantle > gills > adductor muscle > viscera and kidney. The viscera and kidney did not practically release Cd within 672 hours, suggesting a strong binding. The high mortality of Cd-treated mussels during this depuration period can, possibly be connected with this phenomenon.

Our results support these findings, showing that there is a release of Hg and Cd from mussels (Cunningham and Tripp 1973, Fowler et al. 1978). However, this is not the same concerning

the two metals and is different especially for the gills, kidney and viscera.

The results offer a practical guideline for using mussels for monitoring a steady or fluctuating Hg and Cd pollution. Due to the high concentration capacity the best organs for monitoring a steady mercury pollution of the water are the kidney and gill, while in case of Cd pollution kidney and viscera can be recommended. In case the concentration of heavy metals fluctuates in the water, the drop of mercury can be monitored in the kidney, while that of cadmium in the mantle and gills. Due to the low rate of depuration in metal-free water, the gills will reflect mercury pollution for a long time, while for cadmium the kidney can be used for this purpose. These specificities should be taken in consideration when mussels are translocated into a new environment for studying the Hg and Cd pollution in a timely manner.

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DISCUSSION

SHIBER J G: Was the experimentation all conducted in Balaton water? Was the water filtered in order to reduce any possible interference from debris, microorganisms, etc.?

SALÁNKI J: The Balaton water we use in the laboratories is not filtered, only sedimentation of mud takes place in the containers which are at the top of the Institute, where the water is pumped up directly from the Lake. Our intention was to maintain those feeding conditions for the animals.

THEEDE H: The two-phase uptake of heavy metals is an interesting phenomenon. What happens after the first hour of uptake? If there is a preliminary release, e.g. of mercury, what explanation can be given to this? Does this two-phase phenomenon also occur at very low levels of contamination?

SALÁNKI J: The fast uptake and release during the first hours of exposure refers to an active mechanism in the animal which tries to eliminate heavy metals. Certainly at the beginning of the exposure the heavy metals are taken up but are not bound in the tissues, therefore they are eliminated. The slow uptake mechanism may also function already at the first hours, but the stored metal is still very low. Later this second phase predominates, and the metals taken up by this mechanism cannot be easily eliminated.

THEEDE H: Can you give some additional information about the experimental conditions and the food supply during the longterm courses of activity registrations of mussels? Availability of food will be a very decisive factor for the activity pattern.

SALÁNKI J: We did not measure the food content of the water and did not complement the food available in the Balaton water. Algae, microbes and some part of detritus were present but those were absent which were too heavy to sediment in the mud.

THEEDE H: During the depuration phase a release of mercury from different organs was described; only the kidney continued to accumulate. What happens with the hepatopancreas?

In our experiments with Mytilus edulis it could be shown that a redistribution of cadmium took place after the animals had been transferred into clean sea water subsequent to prior accumulation. Some organs lost part of the cadmium, whereas

kidney and hepatopancreas continued to accumulate. Altogether the whole body burden nearly did not change during the course of some weeks in the case the animals had accumulated this metal from low Cd-concentrations in sea water.

SALÁNKI J: We did not investigate the hepatopancreas separately, but together with the viscera (gut, genital organs, etc.) Your findings on the exhcange of cadmium between kidney and hepatopancreas during depuration experiments are very interesting, and really it could give an explanation for a part of our results. Thank you for your comment, - we shall make experiments to check this possibility.

WACHS B: You will take Anodonta as indicator for Hg and Cd pollution. Why do you choose such very high concentrations in your experiments? Are we sure that the accumulation behaviour of mussels will be the same or similar to these results under field conditions in polluted or strongly contaminated rivers?

SALÁNKI J: The concentrations we used in laboratory experiments is naturally higher than in lakes and rivers, but in some places similar concentrations may appear (e.g. industrial waste waters). We suppose, that both the uptake and release of metals, as well as the reaction of bivalves are similar to that we observed in the laboratory. We intend to conduct experiments with 10-100 times lower concentrations as well, in order to get closer to field situations.

LORCH D: I was gratified to see in your slide that mussels react similarly to algae when mercury is accumulated. With algae we too found first a fast metal (lead) accumulation and after about 1 to 2 h a reduction in metal concentration followed by a long term lead accumulation.

How does the total cadmium content of the animal behave during depuration?

SALÁNKI J: We did not measure the total Cd content, since the animals were rather large (wet weight of one specimen is about 100-120 g), but one could calculate it on the basis of the weight of the organs. Since there was a reduction in all organs during depuration (except the kidney), there was obviously release of Cd from the soft body.

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INTERACTIONS OF HEAVY METALS AT TISSUE AND CELLULAR LEVEL IN AQUATIC ORGANISMS

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There is much current concern about the increasing concentrations of heavy metals in both inland and coastal waters resulting from increased industrial activity such as mining (e.g. see Bryan, 1979). Acid rain has also been shown to be responsible for the increase in heavy metal concentrations in a number of freshwater environments (e.g. see Henricksen and Wright, 1978; Wittman, 1981). This is mainly due to metal-containing air-borne particulate matter in the form of rain or snowfall. Also of importance is the resultant increased solubility of bound metal in acid lakes in comparison to neutral waters in the adjoining areas.

As a result of concern towards the aquatic environment, much interest has been shown in the biological and toxicological effects of heavy metals in a number of aquatic organisms. Numerous studies with heavy metals (Fig. 1) have demonstrated their toxic effects (Martin et al., 1981); bioaccumulation (Bryan, 1979; Simkiss et al., 1982); excretion (Simkiss et al., 1982) and binding to e.g. metallothioneins (Roesijadi, 1980). However, little information is available in the literature concerning the mechanism of action of either lethal or sublethal levels of heavy metals before being immobilized or excreted by the organism (Fig. 1). This contribution delineates three physiological effects brought about by copper, but not by zinc, which depend both on the nature of the cell as well as its particular physiological status:

(1) SIPHONAL TISSUE CONTRACTION IN THE MARINE BIVALVE MOLLUSC SCROBICULARIA PLANA

The use of estuarine bivalve molluscs, e.g. Scrobicularia plana and Mytilus edulis, as possible indicator organisms for environmental pollution has been suggested (e.g. see Phillips, 1977; Bryan, 1979). The first visible response occurring when marine bivalve molluscs are exposed to lethal levels of heavy metals is withdrawal of the siphons which is then followed by valve closure (Akberali and Black, 1980; Akberali et al., 1981). Application of both copper and zinc to in situ siphonal tissue of Scrobicularia leads to siphon contraction (Akberali et al., 1981). However, application of comparable zinc concentrations has no apparent effect on the isolated siphonal tissue (Fig. 2). Removal of zinc followed by application of copper results in contraction/relaxation of the isolated siphon with the siphon remaining in a contracted state. These results indicate that copper and zinc have different modes of action on the isolated siphonal tissue

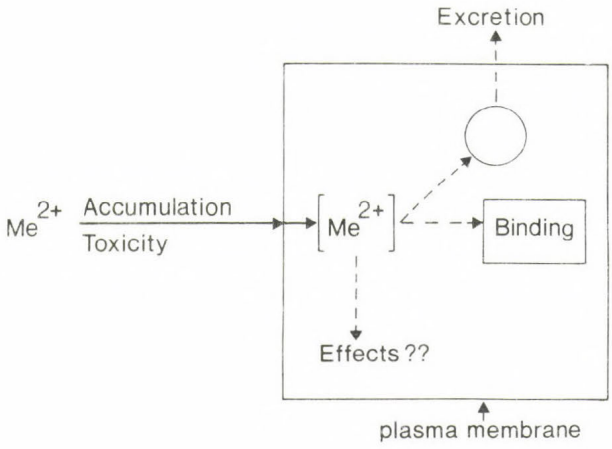

Fig. 1. A schematic representation of an animal cell illustrating various aspects of heavy metal studies. The physiological effects of heavy metals will be discussed in this paper.

preparation in Scrobicularia. It has also been shown that the action of copper on the isolated siphonal tissue is strongly dependent on the presence of calcium in the external bathing medium (Akberali et al., 1982). Furthermore, Akberali (1981) has shown that repeated application of low copper concentrations to the isolated siphonal tissue results in a faster and stronger response which is indicative of a facilitative or cummulative effect.

The effects of the following metal ions, copper, zinc, cadmium, nickel, manganese, chromium (tri or hexavalent), silver and mercury have been tested by Black (1983) on the isolated siphonal tissue in Scrobicularia. From the above series, only copper was effective in eliciting a response in the isolated siphon and at present the reason for this is not clear but may be due to the potent effect of copper on membrane permeability to calcium (see later). Recently, we have also tested the effects of copper on a number of other contractile tissues, e.g. isolated heart of the land snail Helix aspersa, mammalian atria, uterus and diaphram. These studies have also shown that low copper concentrations induce sustained tonic contractions in these tissues (in preparation).

What then is the basis of the differential mode of action of copper and zinc on the isolated molluscan and mammalian contractile tissues? It is possible that the contraction of mollusc siphon (Fig. 2) and other contractile tissues (in preparation) induced by copper may be due to an indirect effect of copper which increases the free cytosolic calcium ion concentration by releasing calcium ion from intracellular calcium reservoirs such as mitochondria, endoplasmic and sarcoplasmic reticulum. Cellular

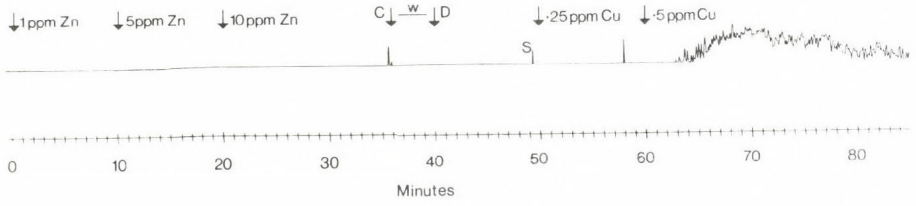

Fig. 2. Effects of various zinc and copper concentrations on an isolated inhalant siphonal tissue preparation of Scrobicularia plana. Upward deflection of the trace indicates isotonic contraction of the inhalant siphonal tissue. The following experimental protocol was carried out in sequence: application of zinc at the final concentrations indicated (↓); removal of seawater containing 10 ppm zinc (C); followed by washes (w) and replacement with normal seawater (D). Prior to copper applications (↓), the siphonal response was tested by a mechanical stimulus (S). From Akberali et al. (1981)

organelles, such as mitochondria, are known to act as regulators of intracellular calcium ion concentration (Bygrave, 1977, Carafoli and Crompton, 1978a). Moreover, mitochondria are thought to play a role in calcium movements during excitation contraction coupling in muscle cells (Huddart and Price, 1976; Carafoli and Crompton, 1978a), which may be particularly important in molluscan smooth muscle in the absence of an organised sarcoplasmic reticulum (Huddart et al., 1977). Alternatively, copper may induce calcium ion efflux from mitochondria (Carafoli and Crompton, 1978b) or smooth endoplasmic reticulum (Blaustein et al., 1978) in the presynaptic nerve terminal producing an increase in intracellular calcium ion activity which triggers transmitter release (Katz and Miledi, 1965; Shapiro et al., 1980).

A tenable hypothesis for the copper-induced tissue contraction is, therefore, that copper releases calcium ion from the intracellular calcium reserves such as in the mitochondria or possibly from another cellular site. The following experiments were designed to shed some light on this hypothesis. Mitochondria were isolated from the digestive gland of Mytilus edulis and the transport of 45 calcium across the inner mitochondrial membrane was measured (Fig. 3). The initial uptake of calcium is rapid and reaches a stable level within 2 min. Addition of copper after 2 min to mitochondria which had been pre-loaded with 45calcium cause a rapid 45calcium efflux resulting in a loss of about 40% at the end of the 5 min efflux period. On the other hand, the 45calcium uptake in the control remained at a relatively stable level over the same experimental period (Fig. 3). In contrast, zinc is relatively ineffective in causing a rapid 45calcium efflux in mitochondria (Akberali and Earnshaw, 1982a). Recent studies with sarcoplasmic reticulum vesicles isolated from mammalian skeletal muscle has shown that a number of heavy metals induce a rapid calcium release from these vesicles (Abramson et al., 1983). These authors have shown that copper is most potent in causing calcium release and have suggested that this may be caused by a dramatic increase in the calcium permeability of the sarcoplasmic reticulum.

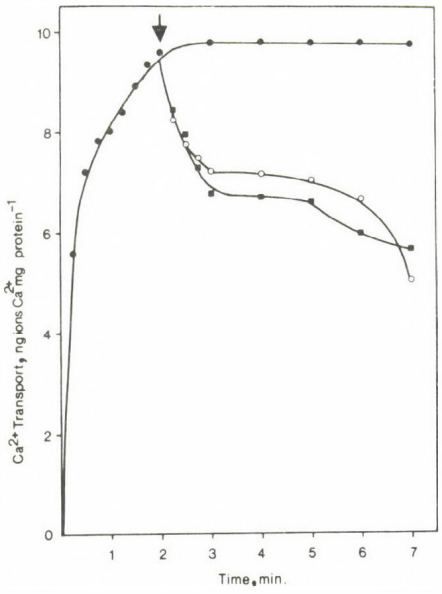

Fig. 3. The effect of copper on accumulated mitochondrial ^{45}calcium. Mitochondria were isolated from Mytilus edulis digestive gland. Mitochondrial calcium transport was determined at 5°C using ^{45}calcium. Copper was added at 2 min (↓) at concentrations of 100 µM (○) and 200 µM (■) and (●) represents calcium transport in the control. From Akberali and Earnshaw (1982a).

It is, therefore, reasonable to suppose that the copper-induced contractions in molluscan and mammalian tissues examined so far is due to an increase in the free cytosolic calcium ion concentration resulting from an intracellular effect of copper.

(2) STIMULATION BY COPPER OF UNFERTILIZED EGG RESPIRATION IN THE MARINE BIVALVE, MYTILUS EDULIS

The effects of copper and zinc in relation to respiration have also been examined using gametes of the marine bivalve Mytilus edulis. The rationale behind this approach is to use the gametes as working models of cellular interactions of heavy metals in order to overcome some of the complexities of working at the organismal level. Furthermore, these studies provide some indication of mechanisms which may be involved in the observed effects of heavy metals on the reduced reproductive potential in aquatic organisms. For example, it is well known that the gametogenic cycle in bivalves, e.g. Mytilus edulis, can be affected by variation in natural environmental parameters such as temperature and food abundance (Bayne et

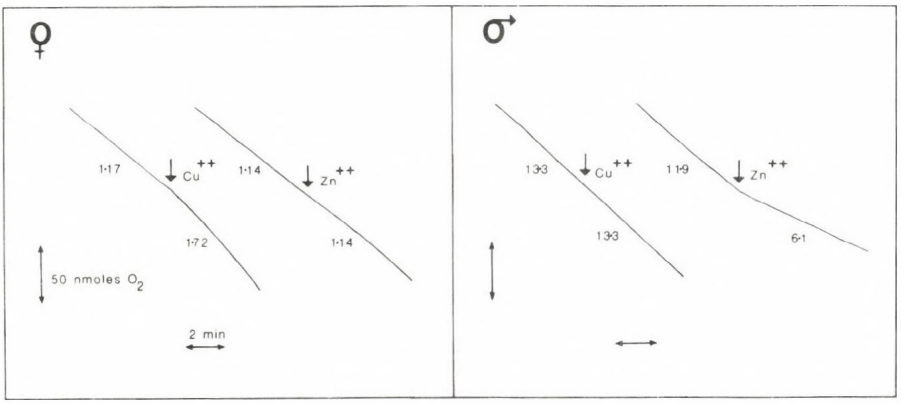

Fig. 4. Oxygen electrode traces showing the effect of direct additions (↓) of copper or zinc on egg and sperm respiration in filtered seawater at 10°C. Heavy metal ions were present at a final concentration of 0.5 mM. Numerals refer to respiration as nmoles oxygen per min per mg protein. From Akberali et al. (1984).

al., 1978) but recent work has shown that continuous exposure to sublethal levels of copper and zinc suppresses gametogenesis in Mytilus edulis, with copper being more toxic (Maung-Myint and Tyler, 1982). Studies on the lethal effects of heavy metals have also demonstrated that embryonic development and larval growth is inhibited in a number of bivalve molluscs (Brereton et al., 1973; Calabrese et al., 1977). Furthermore, the transfer of heavy metals from the gonadal tissues of female bivalve molluscs to their eggs during gametogenesis has also been reported (Greig et al., 1975).

The effects of direct addition of both copper and zinc on sperm and egg respiration are displayed in Figure 4. It is evident that copper has a marked stimulatory effect on egg respiration with no effect on sperm respiration. On the other hand, zinc has no effect on egg respiration but sperm respiration is inhibited. Pre-incubation with the heavy metal for 20 min before measurement of respiration results in inhibition of egg respiration by zinc and sperm respiration by copper (in preparation). It, therefore, seems that the time-dependent permeation of the heavy metal is variable but appears to be more rapid in the reaction of copper with the egg and zinc with the sperm.

The mode of action of copper and zinc on gamete respiration is possibly related to the action of these metal ions on mitochondrial respiration. The respiration of mitochondria isolated from Mytilus digestive gland and mantle tissue is inhibited by zinc (Akberali and Earnshaw, 1982b) suggesting that the inhibition of sperm respiration by zinc (Fig. 4) may be due to its inhibitory effect on mitochondrial respiration. On the other hand, addition

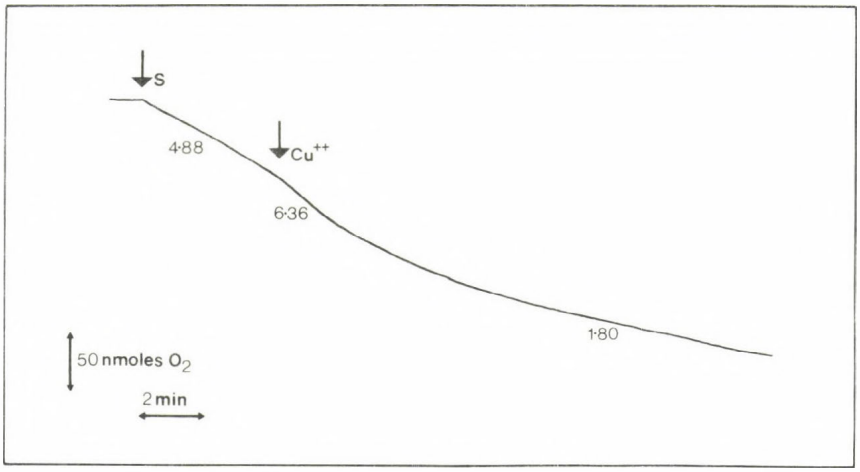

Fig. 5. Oxygen electrode trace showing the action of copper on the respiration of mitochondria isolated from M. edulis mantle tissue. The substrate addition (↓ S) was made 1 min after addition of the mitochondria to the potassium chloride reaction medium and consisted of 6 mM succinate plus 6 mM glutamate. Copper (↓) was added at a final concentration of 0.4 mM. Numerals refer to respiration as nmoles oxygen per min per mg mitochondrial protein. From Akberali et al. (1984).

of copper to respiring Mytilus mantle mitochondria in a reaction medium containing potassium chloride results in an initial stimulation of respiration which is then followed by a progressive inhibition (Fig. 5). Zaba and Harris (1976) have explained the biphasic action of copper on mitochondrial respiration in terms of potassium ion uptake and accompanying mitochondrial swelling leading to respiratory stimulation which becomes progressively inhibited with time at high copper concentrations. It is, therefore, reasonable to assume that a similar situation occurs in Mytilus mitochondria in a reaction medium containing potassium chloride (Fig. 5), particularly as the addition of copper to Mytilus mitochondria in a reaction medium containing sucrose only, does not produce a respiratory stimulation (Akberali and Earnshaw, 1982a).

The stimulation of egg respiration by copper (Fig. 4) indicates that egg respiration is only partially released and the experiment depicted in Fig. 6 shows that it is not subject to substrate limitation since the addition of an uncoupling agent (CCCP) of oxidative phosphorylation results in a marked stimulation of respiration. The uncoupling effect of CCCP on unfertilized egg respiration indicates that the respiration is dampened and this may be due to a high intracellular ATP/ADP ratio which would result in an inhibition of mitochondrial state 3 oxidation. Presumably, the low rate

of basal respiration in the unfertilized egg ensures that the substrate reserves are not depleted prior to fertilization. Fertilization is followed by dramatic increases in, for example, nucleic acid and protein biosynthesis (Balinsky, 1981) which would be expected to reduce the cytosol ATP/ADP ratio which would then lead to the release of respiration. By contrast, sperm possess a reduced potential for uncoupling in the presence of CCCP (Fig. 6) presumably due to the utilization of ATP by the motility mechanism leading to a lower ATP/ADP ratio than in the unfertilized egg.

Therefore, the mode of action of copper in stimulating unfertilized egg respiration (Fig. 4) is most likely due to the uncoupling of mitochondrial respiration at low intracellular copper concentrations (Fig. 5). In contrast, the lack of effect of direct copper addition on sperm respiration (Fig. 4) is presumably, due to the lower uncoupling potential of respiration (Fig. 6).

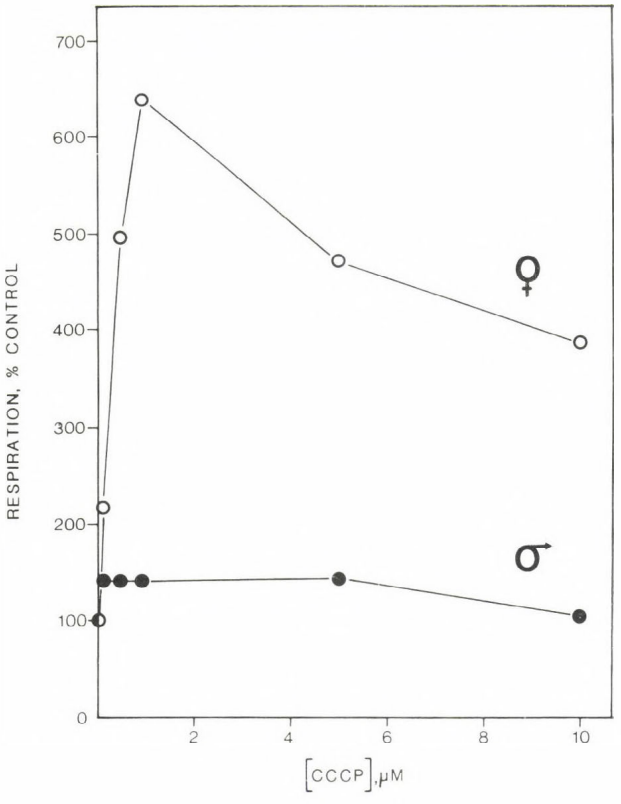

Fig. 6. Egg and sperm respiration as a function of the concentration of carbonyl cyanide m-chlorophenylhydrazone (CCCP). The data represents the mean of two experiments in each case. Control values (100%) were 1.98 nmoles oxygen per min per mg protein for egg respiration and 11.1 nmoles oxygen per min per mg protein for sperm respiration. Conditions as in Fig. 4. Akberali et al. (1984).

(3) STIMULATION BY COPPER OF UNFERTILIZED EGG RESPIRATION IN THE EURASIAN PERCH, PERCA FLUVIATILIS

Over the past few decades, rapid extinction of fish populations inhabiting freshwater habitats affected by acid rain has been observed (Schofield, 1976). These studies clearly indicate that extinction is often a result of chronic reproductive failure due to acid-induced effects on sensitive developmental stages and disruption of reproductive physiology in maturing female fish. Similarly, lethal effects on fish due to an increase in heavy metal concentration have been reported (Schofield and Trojnar, 1980). It has also been shown that in the fish Dicentrarchus labrax, the eggs are more sensitive to copper than are the later developmental stages (Cosson and Martin, 1981).

It is worth pointing out that the uncoupling of Mytilus unfertilized egg respiration by copper is not true for all unfertilized eggs. For example, unfertilized egg respiration in the Eurasian perch, Perca fluviatilis is also stimulated by copper but occurs through a different mechanism. In the perch, addition of copper results in a marked stimulation

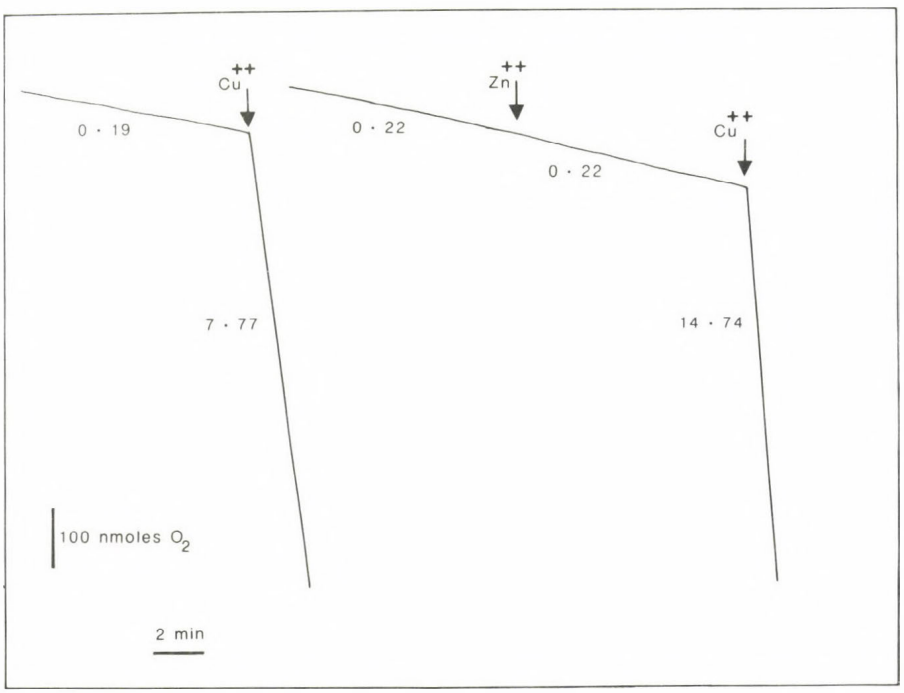

Fig. 7. Oxygen electrode traces showing the stimulation in perch egg respiration caused by copper and the lack of effect of zinc. Arrows (↓) indicate additions of stock copper and zinc solutions to give a final concentration of 0.5 mM. The numerals refer to oxygen uptake as nmoles oxygen per min per mg egg protein at 10°C in filtered pond water. From Akberali and Earnshaw (1984).

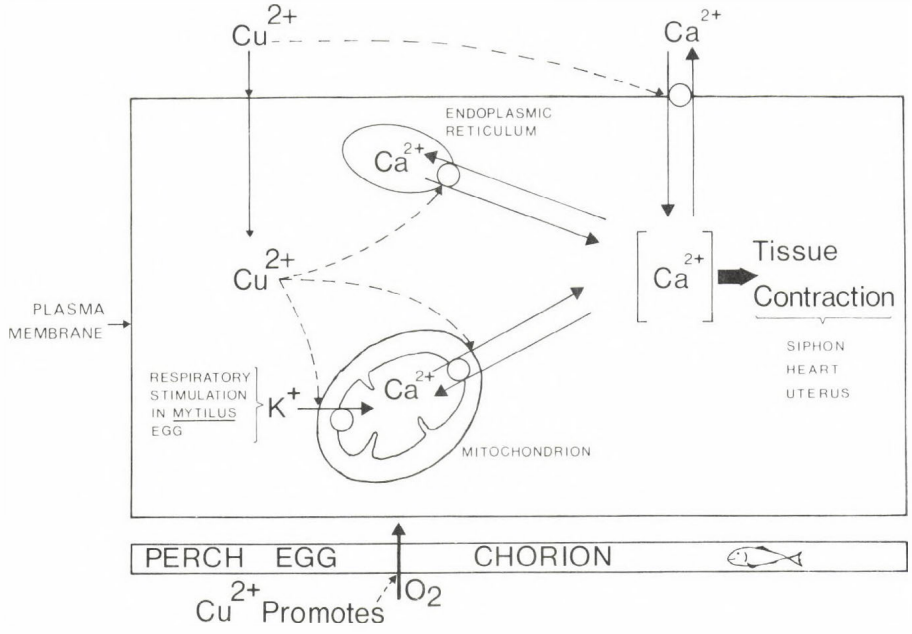

Fig. 8. A stylised model of an animal cell to summarise the possible modes of action of copper on various biochemical and physiological cellular processes. The model illustrates the postulated extracellular and intracellular effects of copper in promoting both the inward calcium conductance current and efflux of calcium from cellular organelles. This will lead to an increase in free cytosolic calcium ion concentration which elicits tissue contraction. In the unfertilized egg of Mytilus edulis, the stimulation of mitochondrial potassium ion uptake results in the uncoupling of egg respiration. In Perca fluviatilis, copper stimulates unfertilized egg respiration by breaking down the oxygen permeability barrier located at the chorion.

(ca 4000%) of the intial respiration rate (Fig. 7) whereas a similar addition of zinc has no effect. In the perch, uncouplers such as CCCP release egg respiration by only ca 60% (Akberali and Earnshaw, 1984) as opposed to 600% in Mytilus (Fig. 6) suggesting that copper-induced stimulation of perch egg respiration occurs via a different mechanism and not through the uncoupling of respiration as in Mytilus eggs. It has been previously suggested (Volodin, 1956; Davenport and Lönning, 1980) that in the teleost egg, gaseous exchange is hindered due to the presence of the chorion and that late stage embryos exist in a low oxygen tension environment. It is, therefore, possible that the copper induced stimulation of respiration in perch egg is due to the removal of oxygen permeability

barrier by copper (Fig. 7). This was examined by determining the effect of temperature on perch egg respiration in the absence and presence of copper (Akberali and Earnshaw, 1984). Surprisingly in the absence of copper, temperature had a little effect on respiration with a Q_{10} (10°-20°C) of 1.06. In contrast, the more normal temperature relationship was observed with the copper-stimulated respiratory rate with a Q_{10} (10°-20°C) of 1.86. From the evidence so far, it is likely that perch egg respiration in the absence of copper is rate-limited by a physical event and that copper is acting by breaking down the oxygen permeability barrier located at the chorion.

(4) RÉSUMÉ AND WORKING MODEL

The mode of action of copper and subsequent effects can be summarized using the model of an animal cell presented in Figure 8. It is postulated that tissue contraction induced by copper arises as a result of an increase in the free cytosolic calcium ion concentration following the interaction of copper with two different membrane systems. Firstly, recent work with the mammalian uterus has shown that copper has an effect at the plasma membrane which results in an increase in the inward extracellular calcium conductance current (in preparation). Secondly, in vivo (in preparation) and in vitro (Akberali and Earnshaw, 1982a) studies suggest that the intracellular entry of copper exerts an effect by promoting calcium ion efflux from mitochondria and endoplasmic/sarcoplasmic reticulum calcium reservoirs. This calcium ion efflux will also contribute to the increase in free cytosolic calcium ion concentration. Therefore, as viewed at present, the effect of copper in promoting spontaneous contractions in a range of contractile tissues, e.g. siphon, heart, uterus, may be the result of an increase in the free cytosolic calcium ion concentration which then induces excitation/contraction coupling in muscle cells or triggers transmitter release in nerve terminals.

In Mytilus, unfertilized egg respiration is only partially released and copper has an uncoupling effect on mitochondrial respiration possibly by initiating potassium ion uptake which will consume energy and hence stimulate egg respiration. In perch eggs, copper appears to promote oxygen entry by breaking down the permeability barrier located at the chorion which results in the stimulation of respiration. The stimulation of unfertilized egg respiration in Mytilus edulis and Perca fluviatilis prior to fertilization by copper will produce significant perturbations in the metabolite pools and in both cases will presumably lead to a decrease in the total substrate reserves which could well affect egg development upon fertilization. By extrapolation, it is also likely that a similar reaction of copper with the gonadal tissue would lead to disruption of the gametogenic cycle. Clearly, the type of cellular perturbation brought about by copper under a given set of circumstances (Fig. 8) will be determined by the nature of the cell as well as its physiological status at the time.

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THE EXCRETION OF HEAVY METALS BY FISH

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SUMMARY

In vertebrates heavy metals are eliminated predominantly by the kidney and/or by the bile. The excretion via urine is the most important route of excretion in mammals. However, Ag, As, (Cd), Cu, Fe, Mn, Pb, (Zn), Zr, and organo-heavy metals are excreted mainly through the bile in dependence on species. Increased concentrations of non-essential heavy metals in urine and bile indicate a contamination caused by the metals in question. The concentration of essential heavy metals in the bile is determined by various factors, e.g. nutrition, stress, intoxication (by organic and inorganic substances), infections. It demonstrates intact or non-intact homeostasis. Considering the specific physiology in the case of essential heavy metals, further studies should test the usability of fish-bile for monitoring environmental toxicants and for detection of sublethal intoxications as far as heavy metals are concerned.

INTRODUCTION

The accumulation of a great deal of environmental pollutants including heavy metals in the organism is increasingly utilized to monitor and detect intoxications. An expert interpretation of findings relating to this is only possible when taking into consideration toxicokinetic aspects. Special attention must be paid to the dose-time-dependences when distributing the sub-

stance in consideration to the individual tissues and organs (Grahl 1983, 1984; Hallebach 1980, 1983). Numerous findings are known concerning the distribution and accumulation of heavy metals in vertebrates. Contrary to this, only a few investigations deal with excretion. It has hardly been noticed that statements concerning excretion may yield essential information on contamination and intoxication, especially of fish. Investigations on fish urine and faeces are utilized in human medicine.

ABSORPTION, TRANSPORT, DISTRIBUTION, BIOTRANSFORMATION AND ACCUMULATION OF HEAVY METALS

Excretion is closely associated with absorption, transport, distribution, biotransformation and accumulation and cannot be considered without dealing with them.

Depending on the way of exposure heavy metals are adsorbed, first of all, at the mucosae (gill, lungs, intestinal mucous membrane, skin of fish and amphibians). Depending on their power of penetrating the mucosae, the following distribution is effected via the systemic circulation. The following factors possess priority: portion of distribution in the blood plasma, portion of distribution in interstitial and intercellular liquids, rate of organ and perfusion, permeability of cell membrane. Particularly important are the availability and the turnover rate of intercellular ligands.

On the other hand, metal-protein bindings in plasma, in the erythrocytes and in various tissues or organs lead to a distribution depending on the possibilities of available bindings. The model of protein binding can greatly vary. Metallothionines are of particular importance since in the systemic liquids of the body there are free ionic species, the distribution of the individual heavy metals occurs in the whole organism.

The essential mechanisms are as follows.

Formation of metal carbon bindings, decomposition of metalcarbon bindings, changes in the state of oxidation of a metal in the biological system. Normally, the biotransformation leads to detoxification, e.g. in the case of arsenic and alcylmercury.

More toxic metabolities are possible intermediates, e.g. the formation of triethyltin from tetraethyltin.
As a result of the distribution and biotransformation as well as depending on excretion, an accumulation of heavy metals in the various tissues and organs may take place. Thereby the accumulated quantity and also the time of accumulation depend on the kind of metal (partially detoxified) in the kidney, liver and spleen. Metal-organic compounds may be accumulated in higher quantities in the brain, too (Alexander 1983; Benton 1984; Camner et al., 1979; Doull et al., 1980; Friberg et al., 1979; Komsta-Szumska et al., 1983a,b,c; Langard 1981, 1982; McKarter et al., 1982; Miettinen 1975; Norseth et al., 1982; Roch et al., 1982; Shinogi et al., 1982). Numerous models of absorption, distribution and accumulation of heavy metals in vertebrates have been presented. Applying these models for monitoring by using the fish test was demonstrated by the example of mercury /Hallebach 1980, 1983/.

EXCRETION OF HEAVY METALS

In vertebrates heavy metals are excreted predominantly by the kidneys and/or by the bile. The excretion via urine is the most important route of excretion in mammals. However, Ag, As, (Cd), Cu, Fe, Mn, Pb, (Zn), Zr and organo-heavy metals are excreted mainly by the bile depending on the species. Of relatively less importance are: pancreatic, intestinal, gonadal and salivary excretions, perspiration, exhalation, lactation, exfoliation of skin, elimination via hair and nails. Data are available on the secretion of essential heavy metals via pancreatic fluid, e.g. Zn, Cu, Mn.With bile investigations being relatively time--consuming, the gastrointestinal excretion is often determined experimentally. Such findings contain beside the biliary excretion the not exactly definable parts of metal excretion via the intestinal mucosa and the pancreatic fluid reduced by the part of reresorption enclosed in the enterohepatic circulation. In the case of oral uptake of heavy metals and without knowing the

amount of heavy metals not resorbed in the intestinal tract, the interpretation of the gastrointestinal excretion becomes doubtful.

Irrespective of the large number of cases in which dominantly renal excretion of heavy metals occurs, nearly all heavy metals as well as some (investigated) heavy metal-organic compounds have been detected. Particularly the following heavy metals are to be mentioned: Ag, As, Cd, Co, Cu, Cr, Fe, Hg, Mo, Mn, Ni, Pb, Sb, Se, Sn, Te, Zn, Zr (Alexander 1983; Camner et al. 1979; Cherian and Vostal 1977; Cikrt 1972; Cikrt and Benko 1979; Cikrt and Tichy 1974; Cikrt et al. 1974; Doull et al. 1980; Grace and Gooden 1980; Hall and Symonds 1980; Havrdova et al. 1974; Iwai et al. 1982; Klaassen 1974, 1976, 1979a; Klaassen and Shoeman 1974; Marafante et al. 1984; Norseth et al. 1982; Refsvik 1978; Schneeman et al. 1983; Strain et al. 1974; Symonds and Hall 1983; Symonds et al. 1983a,b; Tichy 1973; Tichy et al. 1975).

In the fish-bile the following heavy metals could be detected; the maximum concentrations are indicated in brackets: As (159 mg l^{-1}), Cd (0.04 mg l^{-1}), Co (7.5 mg l^{-1}), Cr (2.5 mg l^{-1}), Cu (7.0 mg l^{-1}), Hg (5.0 mg l^{-1}), Ni (11 mg l^{-1}), Pb (26 mg l^{-1}), and Zn (42 mg l^{-1}). These maximum concentrations are to be regarded as more or less incidental values. The concentrations of heavy metals could be found around the concentrations in the kidney, liver and spleen, or they were somewhat higher (Kittelberger 1973; Sorensen et al. 1979).

The majority of analytic determinations of heavy metals in the urine and the bile were made exclusively by atom-absorption spectrometry. This method does not give any answer to the special form of excretion. Only by using fractionation, e.g. permeation chromatography, lower and/or higher molecular species of metals could be detected. These metal species are not always clearly definable conjugates of amino acids or peptides containing thiol (e.g. cysteine, and glutathione). Furthermore, not always clearly definable metal species can be flucuronides and low, medium and high molecular proteins, too (Alexander 1983; Benko 1984; Dukes and Friberg 1972; Norseth et al. 1982; Refsvik and Norseth 1975; Schneeman et al. 1982; Suzuki and Yoshikawa 1981).

In analogy to renal and biliary excretion of xenobiotics, heavy metals predominantly occur in the urine as relatively low molecular compounds. Higher molecular compounds, e.g. metallothioneines, are actually filtered by the glomeruli, but they are reabsorbed in a high degree. This fact explains, among others, the high accumulation of certain heavy metals in the kidney. According to the findings of gel-permeation chromatography, relatively higher molecular compounds exist in the bile. The renal and biliary excretion of heavy metals is influenced by various factors, e.g. chelating agents, inductors and inhibitors of monooxigenases or mixed-function oxidases, interaction of various heavy metals, change in the acid-base status, intoxications, infections, invasions, nutrition and stress. The interpretation of analytic findings relating to the excretion of heavy metals requires the differentiation between essential and non-essential heavy metals. The concentration of the essential heavy metals, such as Cu, Zn and Fe, is influenced, to a great extent, by the factors mentioned above. Disrupted homoeostatis can be characterized by concentration changes of essential heavy metals. Increased concentrations of essential heavy metals therefore do not indicate in each case, a change of the organism due to the respective metal. Furthermore, the increased excretion of heavy metal considered in sublethal intoxications cannot be detected directly. Increased concentrations of non-essential heavy metals put a significant load on the organism. They can be easily used for monitoring and proving the intoxication by heavy metals (Bonner et al. 1979; Caple and Heath 1978; Charmley et al. 1982; Cherian et al. 1982; Cikrt et al. 1975; Klaassen 1979b, Komsta-Szumska et al. 1983b,c; Stowe 1976; Suzuki and Yoshikawa 1981; Symonds et al. 1981, 1983a; Symonds and Mallinson 1982; Tichy and Cikrt 1976).

The present knowledge on the excretion of heavy metals in fish is absolutely insufficient. However, analogous findings as in the case of higher vertebrates are to be expected. Urine investigations of fish, compared to those of mammals, are indeed highly time-consuming, and they can hardly be made routinely. On the other hand, investigations on bile do not

raise any problems concerning small fish. On the understanding that in fish the biliary excretion generally plays a relatively important role, the use of fish bile for monitoring environmental contaminants and for detecting sublethal intoxications could be extended to heavy metals. When investigating residues of environmental contaminants in the future, bile should be more distinctly considered. Besides, residue studies of the bile are relatively easy and, moreover, the favourable factors of accumulation may also be taken into account. Working up the biliary excretion of essential heavy metals could result in a distinct improvement of the interpretation of findings in the case of sublethal intoxications by Cu and Zn.

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BIOCHEMICAL COMPARTMENTATION OF FISH TISSUES, HEAVY METAL TOXICITY ON TISSUE NON-SPECIFIC PHOSPHOMONOESTERASES IN THREE FISHES

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Abstract - The viscera were compartmentalised in relation to non-specific phosphomonoesterases in Labeo rohita /Ham/, Clarias batrachus /Linn/ and Channa punctatus /Bloch/, that varies phylogenetically, biochemically, nutritionally and growth rate wise. The effects of various concentrations /5, 10, 15 and 20 ppm/ of copper and lead were investigated into on differential distribution of acid and alkaline phosphatase in the various regions of liver, muscle, kidney and gill tissues of the above mentioned 3 fish species. The highest fall in alkaline and rise in acid phosphatase activity was recorded in the middle and posterior regions of the kidney respectively /L. rohita/. In liver, the fall in alkaline and rise in acid phosphatase level was greater in the anterior part of the left and right lobes respectively. However, in muscle, the fall in alkaline phosphatase in the cephalic region and the rise in the thoracic region for acid phosphatase was optimum. In gill tissue the fall in alkaline and the rise in acid phosphatase was greater in the left lobe than in the right one.

In all the three fish species, maximum fall in alkaline and acid phosphatase was recorded in L. rohita then in C. batrachus or C. punctatus. The effects of various concentrations of copper on tissue non-specific phosphomonoesterases were more pronounced than those of lead in the above three fish species studied.

Introduction

The toxicity of copper and lead was studied on a variety of fish species in relation to chronic toxicity /Mount and Stephen, 1969/, longterm exposure, survival, growth and reproduction /McKim and Benoit, 1971/, ventilatory activity, blood oxygen and pH /Sellers and Health, 1975/, olfactory response /Hara et al, 1976/ bioenergetics /Lett et al, 1976/ acute lethal levels /Chapman and Stevens, 1978, James et al, 1980/ oxygen consumption /Singh and Singh, 1979/, and size range /Deshmukh and Marathe, 1980/.

Reports regarding the poisoning effect of copper and lead on fishes in relation to tissue damage, physiological and biochemical parameters /Shaffi 1978, Johnson and Larsson, 1979, Shaffi, 1979, Shaffi et al, 1979/ seem to be in need of more investigations.

However, investigations regarding the effect of heavy metals on the biochemical compartmentation of fish tissues seem not to have been attempted. So, in the present investigation the authors have made an attempt to study the effect of various concentrations of copper and lead /5, 10, 15 and 20 ppm/ on the differential distribution of acid and alkaline phosphatase activity in the various regions of liver, kidney, muscle and gill tissue in Labeo rohita /Ham/, Clarias batrachus /Linn/ and Channa puctatus /Bloch/ and also to probe further the exact site of toxicant action in the above mentioned viscera.

Material and methods

Live, healthy and mature L. rohita, C. batrachus and C. punctatus of 18-20 cm. standard length were obtained locally and acclimatized in the laboratory for a week before they were sacrificed for acid and alkaline phosphatase estimation.

Compartmentation of viscera:

Acid and alkaline phosphatase activity was estimated in

left and right lobes of the liver. Based on its length, it was divided into two parts. The kidney was divided into three parts. The muscle tissue was collected at cephalic, thoracic and caudal regions. These two enzymes were estimated in the left and right lobes of the gills.

Enzyme assays:

The preparation of tissue homogenates, incutation mixtures and other details of acid and alkaline phosphatase assays was described earlier /Shaffi and Jeelani, 1984/.

Preparation of copper and lead concentrations:

Copper sulphate /BDH, India, and lead nitrate BDH, India/ was added to water in glass troughs in required amount in order to maintain the concentrations of copper and lead at 5, 10, 15 and 20 ppm.

Exposure to heavy metals:

Seven species of each fish were subjected to various concentrations /5, 10, 15 and 20 ppm/ of copper and lead for a period of four hours. An equal number of fish species were kept in tap water for four hours and treated as the controlled lot. The experiment was repeated five times.

Statistical analysis:

The experiment was repeated five times and the data were subjected to the "F" test.

Results

The results displayed marked variations recorded in acid and alkaline phosphatase activities in various regions of liver, kidney, muscle and gills in L. rohita, C. batrachus and C. punctatus exposed to different concentrations of copper and lead.

Toxicity of copper:

Higher fall and rise in renal alkaline and acid phosphatase activity was recorded in the middle region, then in the posterior and anterior regions respectively /L. rohita, Tab. 5,6/.

The optimum fall in the alkaline phosphatase and rise in the acid phosphatase were observed in the caudal muscle then in the thoracic and cephalic regions of the body. /L. rohita, Tab. 5,6/.

The maximum fall in the alkaline phosphatase and rise in the acid phosphatase was recorded in the posterior part of the left lobe rather than in the right lobe of the liver /L. rohita Tab. 5,6/.

However, in gill tissue the fall in alkaline phosphatase and rise in the acid phosphatase were recorded in the left and the right lobe respectively /L. rohita, Tab. 5,6/.

Toxicity of lead:

The response of the acid and the alkaline phosphatase levels in the various regions of the kidney, the liver, the muscle and the gills to different concentrations of lead /Tab. 7, 8, 9, 10, 11 and 12/ was similar to that of copper except that the rise in the acid phosphatase was higher in the anterior region of the right lobe than in the posterior region and the left lobe in the liver tissue.

In all the above investigations, the effect of copper on the compartmentation of the acid and the alkaline phosphatases were more pronounced than that of lead. Among these three fishes, L. rohita being a soft and herbivorous fish, registered a higher rise and fall in the acid and the alkaline phosphatase in the various regions of the viscera than C. batrachus /Tab. 3 and 4/ and C. punctatus /Tab. 1 and 2/ which are carnivorous, piscivorous and hard fish species.

Discussion

The heavy metal toxicity is ascribed to the fall in the diffusing capacity of the gill, the decreased oxygen tension, the reduced oxygen consumption, the physiological imbalance, restlessness, the fall in blood pH, the increased gill ventilation, the opercular movement, the breathing rate and the concentration of metabolic products /Chapman and Stevens, 1978, Deshmukh and Marathe, 1980, Hara et al, 1976, Hughes, 1976, Lett et al, 1976, Lloyd, 1961, McKim and Benoit, 1971, Sellers and Health, 1975, Shaffi, 1979, Shaffi et al, 1979, Singh and Singh, 1979/.

Precipitation of the mucus around the gills and the general body surface, shrinkage of respiratory epithelium, and respiratory distress due to various concentrations of copper and lead toxicity might lead to the breakdown of gas exchange at the gills /Shaffi, 1978, Shaffi, 1979/. Owing to the breakdown of gas exchange, the tissues might be subjected to low levels of oxygen, thus causing tissue hypoxia /Shaffi, 1979, Shaffi, 1981/. This situation may lead to tissue acidosis and it may alter the buffering system of the viscera which might affect further a number of other biochemical constituents.

Heavy metal exposure also causes the arrest of branchial circulation which may reduce the supply of oxygen to the remaining parts of the body /Hughes, 1976/. It seems that this process may lead to increased respiratory rate and finally cause respiratory distress and it may lead to the precipitation of some metabolic products which may affect the functioning of the fish itself /Skidmore, 1970/.

The above mentioned sequence of events may lead to the rapture of cellular and lysosomal membranes which will liberate their constituents by lysis /de Deuve, 1956, Shaffi, 1980/. The rise in acid phosphatase level in the various regions of the kidney, the liver, the muscle and gills may be the outcome of the above process. The fall in the alkaline phosphatase level may be due to tissue acidosis, alteration in buffering system, accumulation of metabolic products, and uncoupling

phosphorylation. The optimum rise in acid phosphatase and fall in alkaline phosphatase in a particular region of the organ might indicate the site of toxicant action in that organ.

Among these three fish species the optimum rise in acid phosphatase and fall in alkaline phosphatase was recorded in L. rohita which absolutely depends on gill respiration rather than in C. batrachus and C. punctatus which can also survive by accessory respiratory system. It appears from this study that hard fishes /C. batrachus and C. punctatus/ are more resistant to toxicants than soft /L. rohita/ fish species. The visceral acid and alkaline phosphatase response to copper is more pronounced than to lead in the three fish species studied and it may be explained on the following lines.

The ions of heavy metals interact with sulphydryl groups of proteins and it may cause the precipitation of protein /Shaffi et al, 1979/. Such a process might have occurred with copper and lead in the present investigation and the precipitation might be higher with copper than with lead. The differential toxicity responses may depend on the accumulation of pollutants, biochemical build-up and the level of phylogeny.

Table 1 - Copper toxicity: Alkaline phosphatase in Channa punctatus

	Control	Concentrations 5 ppm	10 ppm	15 ppm	20 ppm	% of F/R
Kidney						
Anterior	0.532 ± 0.080	0.563 ± 0.094	0.461 ± 0.078	0.392 ± 0.039	0.286 ± 0.031	46.2 F
Middle	0.652 ± 0.095	0.688 ± 0.099	0.460 ± 0.060	0.380 ± 0.042	0.251 ± 0.028	61.42F
Posterior	0.428 ± 0.065	0.450 ± 0.048	0.400 ± 0.037	0.340 ± 0.038	0.201 ± 0.024	53.12F
Muscle						
Cephalic	0.200 ± 0.031	0.210 ± 0.028	0.183 ± 0.020	0.157 ± 0.024	0.124 ± 0.014	38.12F
Thoracic	0.252 ± 0.040	0.230 ± 0.024	0.200 ± 0.017	0.170 ± 0.020	0.132 ± 0.015	47.83F
Caudal	0.300 ± 0.048	0.263 ± 0.030	0.220 ± 0.025	0.180 ± 0.016	0.125 ± 0.015	58.36F
Liver						
Left lobe Anterior	0.282 ± 0.038	0.250 ± 0.030	0.222 ± 0.028	0.194 ± 0.027	0.174 ± 0.020	38.29F
Left lobe Posterior	0.217 ± 0.027	0.200 ± 0.021	0.171 ± 0.015	0.131 ± 0.018	0.106 ± 0.015	51.00F
Right lobe Anterior	0.310 ± 0.041	0.283 ± 0.030	0.260 ± 0.027	0.241 ± 0.023	0.211 ± 0.025	32.03F
Right lobe Posterior	0.229 ± 0.032	0.206 ± 0.025	0.181 ± 0.023	0.154 ± 0.019	0.120 ± 0.020	47.54F
Gills						
Left lobe	0.123 ± 0.016	0.115 ± 0.010	0.097 ± 0.013	0.080 ± 0.010	0.065 ± 0.012	47.00F
Right lobe	0.164 ± 0.021	0.150 ± 0.011	0.143 ± 0.015	0.120 ± 0.010	0.106 ± 0.014	35.24F

Values are mean ± SDM of 6 replicates. /Enzyme activity/ug of Pi/mg protein at 37°C/. "F" Test was performed. "F" is 0.05. Side script "F" and "R" indicates Fall and Rise respectively.

Table 2 - Copper toxicity: Acid phosphatase in Channa punctatus

	Control	Concentrations				% of F/R
		5 ppm	10 ppm	15 ppm	20 ppm	
Kidney						
Anterior	0.332 ± 0.065	0.374 ± 0.038	0.438 ± 0.062	0.498 ± 0.083	0.599 ± 0.095	50.31R
Middle	0.430 ± 0.060	0.471 ± 0.063	0.496 ± 0.074	0.620 ± 0.090	0.722 ± 0.112	67.93R
Posterior	0.260 ± 0.051	0.289 ± 0.042	0.320 ± 0.038	0.351 ± 0.045	0.408 ± 0.063	56.99R
Muscle						
Cephalic	0.163 ± 0.022	0.178 ± 0.019	0.194 ± 0.025	0.212 ± 0.026	0.233 ± 0.024	43.20R
Thoracic	0.221 ± 0.038	0.253 ± 0.031	0.279 ± 0.030	0.311 ± 0.028	0.341 ± 0.029	54.12R
Caudal	0.275 ± 0.041	0.311 ± 0.040	0.341 ± 0.038	0.382 ± 0.032	0.438 ± 0.063	59.25R
Liver						
Right lobe Left lobe Anterior	0.412 ± 0.058	0.425 ± 0.048	0.465 ± 0.060	0.494 ± 0.063	0.554 ± 0.092	34.55R
Right lobe Left lobe Posterior	0.320 ± 0.050	0.351 ± 0.042	0.385 ± 0.040	0.419 ± 0.058	0.472 ± 0.062	48.12R
Right lobe Anterior	0.333 ± 0.028	0.365 ± 0.038	0.412 ± 0.040	0.462 ± 0.061	0.507 ± 0.061	52.38R
Right lobe Posterior	0.400 ± 0.050	0.431 ± 0.040	0.478 ± 0.051	0.500 ± 0.063	0.555 ± 0.070	38.67R
Gills						
Left lobe	0.112 ± 0.014	0.120 ± 0.011	0.131 ± 0.013	0.145 ± 0.016	0.152 ± 0.018	35.29R
Right lobe	0.080 ± 0.012	0.091 ± 0.009	0.099 ± 0.010	0.109 ± 0.012	0.114 ± 0.013	42.00R

Values are mean ± SDM of 6 replicates. /Enzyme activity ug of Pi/mg protein at 37°C/. "F" test was performed. "F" is 0.05. Side script "F" and "R" indicates Fall and Rise respectively.

Table 3 - Copper toxicity: Alkaline phosphatase in Clarias batrachus

	Control	Concentrations				% of F/R
		5 ppm	10 ppm	15 ppm	20 ppm	
Kidney						
Anterior	0.481 ± 0.061	0.500 ± 0.095	0.435 ± 0.082	0.360 ± 0.090	0.240 ± 0.028	50.12F
Middle	0.575 ± 0.093	0.595 ± 0.100	0.500 ± 0.063	0.306 ± 0.065	0.173 ± 0.020	69.90F
Posterior	0.403 ± 0.050	0.412 ± 0.053	0.320 ± 0.031	0.247 ± 0.030	0.172 ± 0.025	57.39F
Muscle						
Cephalic	0.172 ± 0.018	0.150 ± 0.015	0.132 ± 0.018	0.117 ± 0.020	0.099 ± 0.011	42.36F
Thoracic	0.222 ± 0.020	0.200 ± 0.024	0.173 ± 0.019	0.154 ± 0.018	0.107 ± 0.015	51.74F
Caudal	0.270 ± 0.030	0.240 ± 0.029	0.175 ± 0.020	0.120 ± 0.016	0.091 ± 0.014	66.42F
Liver						
Left lobe Anterior	0.240 ± 0.030	0.215 ± 0.013	0.183 ± 0.019	0.150 ± 0.017	0.125 ± 0.015	47.97F
Left lobe Posterior	0.186 ± 0.020	0.170 ± 0.018	0.138 ± 0.015	0.102 ± 0.013	0.085 ± 0.010	55.28F
Right lobe Anterior	0.269 ± 0.030	0.240 ± 0.026	0.202 ± 0.020	0.168 ± 0.025	0.151 ± 0.018	44.00F
Right lobe Posterior	0.170 ± 0.024	0.153 ± 0.017	0.136 ± 0.017	0.099 ± 0.015	0.079 ± 0.014	53.48F
Gills						
Left lobe	0.105 ± 0.017	0.100 ± 0.010	0.093 ± 0.012	0.063 ± 0.007	0.047 ± 0.008	54.82F
Right lobe	0.145 ± 0.020	0.130 ± 0.012	0.113 ± 0.011	0.096 ± 0.012	0.087 ± 0.010	40.00F

Values are mean ± SDM of 6 replicates. /Enzyme activity ug of Pi/mg protein at 37°C/. "F" Test was performed. "F" is 0.05. Side script "F" and "R" indicates Fall and Rise respectively.

Table 4 - Copper toxicity: Acid phosphatase in Clarias batrachus

		Concentrations				% of F/R
	Control	5 ppm	10 ppm	15 ppm	20 ppm	
Kidney						
Anterior	0.234 ± 0.024	0.264 ± 0.031	0.290 ± 0.035	0.315 ± 0.041	0.359 ± 0.063	53.22R
Middle	0.300 ± 0.035	0.340 ± 0.040	0.399 ± 0.039	0.462 ± 0.060	0.523 ± 0.083	74.54R
Posterior	0.169 ± 0.026	0.183 ± 0.019	0.222 ± 0.025	0.248 ± 0.028	0.281 ± 0.034	66.00R
Muscle						
Cephalic	0.166 ± 0.020	0.178 ± 0.035	0.191 ± 0.020	0.212 ± 0.030	0.240 ± 0.035	44.81R
Thoracic	0.200 ± 0.021	0.220 ± 0.028	0.265 ± 0.020	0.293 ± 0.031	0.319 ± 0.039	59.30R
Caudal	0.239 ± 0.025	0.251 ± 0.033	0.296 ± 0.030	0.328 ± 0.026	0.394 ± 0.043	65.00R
Liver						
Left lobe Anterior	0.325 ± 0.054	0.351 ± 0.038	0.385 ± 0.043	0.412 ± 0.051	0.448 ± 0.060	37.56R
Left lobe Posterior	0.248 ± 0.038	0.265 ± 0.020	0.290 ± 0.031	0.315 ± 0.040	0.363 ± 0.051	46.22R
Right lobe Anterior	0.252 ± 0.033	0.278 ± 0.032	0.306 ± 0.045	0.345 ± 0.060	0.399 ± 0.078	58.45R
Right lobe Posterior	0.316 ± 0.048	0.348 ± 0.083	0.380 ± 0.060	0.400 ± 0.060	0.431 ± 0.064	36.39R
Gills						
Left lobe	0.100 ± 0.010	0.113 ± 0.015	0.121 ± 0.014	0.128 ± 0.018	0.143 ± 0.019	43.00R
Right lobe	0.074 ± 0.007	0.086 ± 0.008	0.095 ± 0.009	0.115 ± 0.013	0.134 ± 0.016	48.36R

Values are mean ± SDM of 6 replicates. /Enzyme activity ug of Pi/mg protein at 37°C/. "F" test was performed. "F" is 0.05. Side script "F" and "R" indicates Fall and Rise respectively.

Table 5 - Copper toxicity: Alkaline phosphatase in Labeo rohita

	Control	Concentrations				% of F/R
		5 ppm	10 ppm	15 ppm	20 ppm	
Kidney						
Anterior	0.270 ± 0.036	0.285 ± 0.040	0.224 ± 0.022	0.163 ± 0.018	0.078 ± 0.014	71.05F
Middle	0.345 ± 0.058	0.378 ± 0.062	0.300 ± 0.042	0.155 ± 0.020	0.053 ± 0.012	84.63F
Posterior	0.205 ± 0.021	0.218 ± 0.020	0.162 ± 0.018	0.095 ± 0.012	0.050 ± 0.011	75.44F
Muscle						
Cephalic	0.121 ± 0.015	0.111 ± 0.017	0.100 ± 0.012	0.086 ± 0.012	0.061 ± 0.010	49.39F
Thoracic	0.154 ± 0.018	0.132 ± 0.019	0.114 ± 0.021	0.092 ± 0.010	0.063 ± 0.011	59.00F
Caudal	0.193 ± 0.020	0.145 ± 0.016	0.109 ± 0.025	0.083 ± 0.011	0.052 ± 0.009	72.36F
Liver						
Left lobe Anterior	0.165 ± 0.020	0.144 ± 0.017	0.103 ± 0.020	0.082 ± 0.011	0.056 ± 0.012	66.25F
Left lobe Posterior	0.133 ± 0.016	0.120 ± 0.020	0.089 ± 0.012	0.062 ± 0.014	0.033 ± 0.015	74.99F
Right lobe Anterior	0.175 ± 0.021	0.155 ± 0.028	0.135 ± 0.014	0.099 ± 0.013	0.070 ± 0.009	60.45F
Right lobe Posterior	0.121 ± 0.018	0.100 ± 0.012	0.092 ± 0.015	0.060 ± 0.013	0.034 ± 0.010	72.06F
Gills						
Left lobe	0.072 ± 0.009	0.063 ± 0.010	0.050 ± 0.008	0.040 ± 0.009	0.026 ± 0.008	63.47F
Right lobe	0.096 ± 0.011	0.080 ± 0.009	0.072 ± 0.009	0.063 ± 0.010	0.048 ± 0.005	50.00F

Values are mean ± SDM of 6 replicates. /Enzyme activity ug of Pi/mg protein at 37°C/. "F" test was performed. "F" is 0.05. Side script "F" and "R" indicates Fall and Rise respectively.

Table 6 - Copper toxicity: Acid phosphatase in Labeo rohita

		Concentrations				% of F/R
	Control	5 ppm	10 ppm	15 ppm	20 ppm	
Kidney						
Anterior	0.188 ± 0.28	0.230 ± 0.025	0.256 ± 0.034	0.278 ± 0.041	0.305 ± 0.030	62.00R
Middle	0.244 ± 0.035	0.268 ± 0.035	0.315 ± 0.042	0.370 ± 0.051	0.452 ± 0.055	85.37R
Posterior	0.141 ± 0.018	0.177 ± 0.019	0.195 ± 0.024	0.220 ± 0.025	0.248 ± 0.029	76.11R
Muscle						
Cephalic	0.105 ± 0.014	0.118 ± 0.018	0.128 ± 0.024	0.137 ± 0.020	0.155 ± 0.021	47.33R
Thoracic	0.141 ± 0.015	0.160 ± 0.016	0.189 ± 0.025	0.202 ± 0.031	0.225 ± 0.033	59.25R
Caudal	0.176 ± 0.020	0.183 ± 0.025	0.216 ± 0.030	0.250 ± 0.033	0.303 ± 0.034	72.35R
Liver						
Left lobe Anterior	0.209 ± 0.023	0.225 ± 0.024	0.248 ± 0.029	0.270 ± 0.031	0.303 ± 0.036	44.83R
Left lobe Posterior	0.160 ± 0.021	0.173 ± 0.019	0.192 ± 0.024	0.220 ± 0.030	0.253 ± 0.038	58.22R
Right lobe Anterior	0.160 ± 0.019	0.193 ± 0.021	0.215 ± 0.017	0.226 ± 0.031	0.258 ± 0.041	61.15R
Right lobe Posterior	0.200 ± 0.025	0.223 ± 0.024	0.245 ± 0.0287	0.280 ± 0.031	0.299 ± 0.034	49.55R
Gills						
Left lobe	0.088 ± 0.011	0.096 ± 0.012	0.115 ± 0.013	0.130 ± 0.016	0.150 ± 0.014	70.00R
Right lobe	0.060 ± 0.013	0.071 ± 0.010	0.084 ± 0.013	0.092 ± 0.012	0.109 ± 0.013	81.14R

Values are mean ± SDM of 6 replicates. /Enzyme activity ug of Pi/mg protein at 37°C/. "F" test was performed. "F" is 0.05. Side script "F" and "R" indicates Fall and Rise respectively.

Table 7 - Lead toxicity: Alkaline phosphatase in Channa punctatus

		Concentrations				% of F/R
	Control	5 ppm	10 ppm	15 ppm	20 ppm	
Kidney						
Anterior	0.532 ± 0.080	0.550 ± 0.085	0.485 ± 0.073	0.417 ± 0.080	0.350 ± 0.045	34.22F
Middle	0.652 ± 0.095	0.672 ± 0.093	0.571 ± 0.063	0.436 ± 0.062	0.324 ± 0.034	50.24F
Posterior	0.428 ± 0.065	0.430 ± 0.050	0.362 ± 0.033	0.300 ± 0.028	0.247 ± 0.029	42.28F
Muscle						
Cephalic	0.200 ± 0.031	0.185 ± 0.012	0.172 ± 0.019	0.163 ± 0.013	0.146 ± 0.017	27.04F
Thoracic	0.252 ± 0.040	0.230 ± 0.025	0.211 ± 0.020	0.183 ± 0.020	0.155 ± 0.024	39.55F
Caudal	0.300 ± 0.048	0.280 ± 0.020	0.241 ± 0.028	0.195 ± 0.022	0.155 ± 0.031	48.32F
Liver						
Left lobe Anterior	0.282 ± 0.038	0.260 ± 0.025	0.238 ± 0.042	0.217 ± 0.024	0.191 ± 0.020	32.11F
Left lobe Posterior	0.217 ± 0.027	0.200 ± 0.031	0.184 ± 0.017	0.150 ± 0.019	0.129 ± 0.031	40.57F
Right lobe Anterior	0.310 ± 0.041	0.286 ± 0.034	0.260 ± 0.020	0.145 ± 0.024	0.219 ± 0.028	29.35F
Right lobe Posterior	0.229 ± 0.032	0.200 ± 0.040	0.189 ± 0.030	0.170 ± 0.017	0.142 ± 0.019	38.19F
Gills						
Left lobe	0.123 ± 0.016	0.116 ± 0.020	0.102 ± 0.014	0.093 ± 0.009	0.082 ± 0.012	33.00F
Right lobe	0.164 ± 0.021	0.156 ± 0.023	0.148 ± 0.017	0.142 ± 0.013	0.130 ± 0.010	21.00F

Values are mean ± SDM of 6 replicates. /Enzyme activity ug of Pi/mg protein at 37°C/. "F" test was performed. "F" is 0.05. Side script "F" and "R" indicates Fall and Rise respectively.

Table 8 - Lead Toxicity: Acid phosphatase in Channa punctatus

	Control	Concentrations				% of F/R
		5 ppm	10 ppm	15 ppm	20 ppm	
Kidney						
Anterior	0.332 ± 0.065	0.360 ± 0.035	0.379 ± 0.045	0.400 ± 0.032	0.459 ± 0.060	38.36R
Middle	0.430 ± 0.060	0.460 ± 0.053	0.500 ± 0.057	0.580 ± 0.063	0.676 ± 0.098	57.24R
Posterior	0.260 ± 0.051	0.285 ± 0.044	0.311 ± 0.030	0.330 ± 0.035	0.386 ± 0.038	48.32R
Muscle						
Cephalic	0.163 ± 0.022	0.174 ± 0.019	0.189 ± 0.022	0.200 ± 0.026	0.219 ± 0.031	34.29R
Thoracic	0.221 ± 0.038	0.242 ± 0.030	0.268 ± 0.027	0.290 ± 0.034	0.329 ± 0.035	42.96R
Caudal	0.275 ± 0.041	0.289 ± 0.035	0.340 ± 0.040	0.385 ± 0.031	0.422 ± 0.060	53.44R
Liver						
Left lobe Anterior	0.412 ± 0.058	0.438 ± 0.045	0.462 ± 0.060	0.490 ± 0.053	0.515 ± 0.060	25.00R
Left lobe Posterior	0.320 ± 0.050	0.323 ± 0.040	0.365 ± 0.031	0.395 ± 0.036	0.447 ± 0.054	39.63R
Right lobe Anterior	0.333 ± 0.028	0.368 ± 0.039	0.401 ± 0.030	0.435 ± 0.037	0.487 ± 0.061	46.24R
Right lobe Posterior	0.400 ± 0.050	0.415 ± 0.051	0.430 ± 0.032	0.455 ± 0.050	0.496 ± 0.059	24.00R
Gills						
Left lobe	0.112 ± 0.014	0.120 ± 0.015	0.125 ± 0.013	0.131 ± 0.010	0.146 ± 0.014	30.01R
Right lobe	0.080 ± 0.012	0.086 ± 0.006	0.095 ± 0.010	0.103 ± 0.009	0.112 ± 0.013	39.36R

Values are mean ± SDM of 6 replicates. /Enzyme activity ug of Pi/mg protein at 37°C/. "F" test was performed. "F" is 0.05. Side script "F" and "R" indicates Fall and Rise respectively.

Table 9 - Lead toxicity: Alkaline phosphatase in Clarias batrachus

	Control	Concentrations				% of F/R
		5 ppm	10 ppm	15 ppm	20 ppm	
Kidney						
Anterior	0.481 ± 0.061	0.490 ± 0.063	0.421 ± 0.058	0.365 ± 0.039	0.283 ± 0.021	41.11F
Middle	0.575 ± 0.093	0.595 ± 0.074	0.500 ± 0.065	0.400 ± 0.055	0.240 ± 0.031	58.24F
Posterior	0.403 ± 0.050	0.421 ± 0.068	0.328 ± 0.034	0.271 ± 0.028	0.202 ± 0.020	49.90F
Muscle						
Cephalic	0.172 ± 0.018	0.160 ± 0.018	0.143 ± 0.019	0.130 ± 0.015	0.117 ± 0.020	32.00F
Thoracic	0.200 ± 0.020	0.200 ± 0.025	0.175 ± 0.030	0.143 ± 0.020	0.118 ± 0.014	46.82F
Caudal	0.270 ± 0.030	0.251 ± 0.031	0.200 ± 0.024	0.171 ± 0.015	0.116 ± 0.020	57.18F
Liver						
Left lobe Anterior	0.240 ± 0.030	0.213 ± 0.017	0.189 ± 0.029	0.170 ± 0.019	0.146 ± 0.016	39.22F
Left lobe Posterior	0.186 ± 0.020	0.180 ± 0.021	0.144 ± 0.020	0.120 ± 0.013	0.093 ± 0.011	50.15F
Right lobe Anterior	0.269 ± 0.030	0.240 ± 0.031	0.212 ± 0.025	0.190 ± 0.020	0.174 ± 0.020	35.28F
Right lobe Posterior	0.170 ± 0.024	0.151 ± 0.014	0.134 ± 0.016	0.117 ± 0.024	0.091 ± 0.020	46.66F
Gills						
Left lobe	0.105 ± 0.017	0.098 ± 0.009	0.090 ± 0.010	0.079 ± 0.008	0.065 ± 0.007	38.16F
Right lobe	0.145 ± 0.020	0.131 ± 0.013	0.120 ± 0.011	0.115 ± 0.015	0.108 ± 0.009	25.80F

Values are mean ± SDM of 6 replicates. /Enzyme activity ug of Pi/mg protein at 37°C/. "F" test was performed. "F" is 0.05. Side script "F" and "R" indicates Fall and Rise respectively.

Table 10 - Lead toxicity: Acid phosphatase in Clarias batrachus

		Concentrations				% of F/R
	Control	5 ppm	10 ppm	15 ppm	20 ppm	
Kidney						
Anterior	0.234 ± 0.024	0.261 ± 0.031	0.285 ± 0.042	0.301 ± 0.040	0.336 ± 0.045	43.44R
Middle	0.300 ± 0.035	0.341 ± 0.025	0.378 ± 0.049	0.420 ± 0.062	0.490 ± 0.073	63.59R
Posterior	0.169 ± 0.026	0.183 ± 0.014	0.195 ± 0.021	0.220 ± 0.023	0.260 ± 0.025	53.98R
Muscle						
Cephalic	0.166 ± 0.020	0.178 ± 0.024	0.193 ± 0.027	0.110 ± 0.020	0.222 ± 0.017	33.62R
Thoracic	0.200 ± 0.021	0.216 ± 0.020	0.229 ± 0.021	0.242 ± 0.031	0.280 ± 0.035	40.00R
Caudal	0.239 ± 0.025	0.265 ± 0.024	0.289 ± 0.035	0.315 ± 0.030	0.363 ± 0.035	52.00R
Liver						
Left lobe Anterior	0.325 ± 0.054	0.340 ± 0.045	0.371 ± 0.051	0.394 ± 0.040	0.428 ± 0.040	30.19R
Left lobe Posterior	0.248 ± 0.038	0.268 ± 0.028	0.291 ± 0.030	0.315 ± 0.020	0.350 ± 0.034	41.35R
Right lobe Anterior	0.252 ± 0.033	0.274 ± 0.024	0.283 ± 0.020	0.321 ± 0.036	0.364 ± 0.040	44.29R
Right lobe Posterior	0.316 ± 0.048	0.340 ± 0.038	0.365 ± 0.035	0.383 ± 0.045	0.413 ± 0.061	30.72R
Gills						
Left lobe	0.100 ± 0.010	0.112 ± 0.015	0.116 ± 0.011	0.120 ± 0.013	0.128 ± 0.015	28.00R
Right lobe	0.074 ± 0.007	0.078 ± 0.012	0.086 ± 0.009	0.095 ± 0.009	0.102 ± 0.011	37.44R

Values are mean ± SDM of 6 replicates. /Enzyme activity ug of Pi/mg protein at 37°C/. "F" test was performed. "F" is 0.05. Side script "F" and "R" indicates Fall and Rise respectively.

Table 11 - Lead toxicity: Alkaline phosphatase in Labeo rohita

	Control	Concentrations				% of F/R
		5 ppm	10 ppm	15 ppm	20 ppm	
Kidney						
Anterior	0.270 ± 0.036	0.281 ± 0.032	0.239 ± 0.028	0.185 ± 0.028	0.129 ± 0.020	52.33F
Middle	0.345 ± 0.058	0.360 ± 0.051	0.300 ± 0.050	0.240 ± 0.042	0.104 ± 0.015	70.00F
Posterior	0.205 ± 0.021	0.220 ± 0.022	0.165 ± 0.020	0.136 ± 0.019	0.082 ± 0.010	59.99F
Muscle						
Cephalic	0.121 ± 0.015	0.111 ± 0.015	0.099 ± 0.015	0.086 ± 0.009	0.070 ± 0.011	42.33F
Thoracic	0.154 ± 0.018	0.140 ± 0.017	0.112 ± 0.011	0.098 ± 0.010	0.075 ± 0.012	51.47F
Caudal	0.193 ± 0.020	0.173 ± 0.020	0.140 ± 0.020	0.109 ± 0.015	0.070 ± 0.009	63.66F
Liver						
Left lobe Anterior	0.165 ± 0.020	0.142 ± 0.015	0.119 ± 0.013	0.100 ± 0.012	0.080 ± 0.013	51.55F
Left lobe Posterior	0.133 ± 0.016	0.112 ± 0.016	0.092 ± 0.012	0.080 ± 0.009	0.054 ± 0.009	59.23F
Right lobe Anterior	0.175 ± 0.021	0.149 ± 0.020	0.130 ± 0.019	0.115 ± 0.021	0.091 ± 0.024	48.09F
Right lobe Posterior	0.121 ± 0.018	0.110 ± 0.025	0.095 ± 0.017	0.072 ± 0.012	0.053 ± 0.011	55.93F
Gills						
Left lobe	0.072 ± 0.009	0.064 ± 0.009	0.052 ± 0.008	0.044 ± 0.010	0.039 ± 0.008	45.45F
Right lobe	0.096 ± 0.011	0.090 ± 0.010	0.081 ± 0.009	0.075 ± 0.011	0.066 ± 0.007	31.11F

Values are mean ± SDM of 6 replicates. /Enzyme activity ug of Pi/mg protein at 37°C/. "F" test was performed. "F" is 0.05. Side script "F" and "R" indicates Fall and Rise respectively.

Table 12 - Lead toxicity: Acid phosphatase in Labeo rohita

	Control	Concentrations 5 ppm	10 ppm	15 ppm	20 ppm	% of F/R
Kidney						
Anterior	0.188 ± 0.028	0.215 ± 0.020	0.230 ± 0.024	0.251 ± 0.030	0.274 ± 0.031	45.43R
Middle	0.244 ± 0.035	0.270 ± 0.035	0.301 ± 0.040	0.340 ± 0.025	0.399 ± 0.044	63.55R
Posterior	0.141 ± 0.018	0.156 ± 0.018	0.178 ± 0.020	0.193 ± 0.024	0.219 ± 0.030	55.42R
Muscle						
Cephalic	0.103 ± 0.014	0.117 ± 0.015	0.129 ± 0.024	0.134 ± 0.018	0.145 ± 0.015	38.14R
Thoracic	0.141 ± 0.015	0.160 ± 0.014	0.178 ± 0.020	0.183 ± 0.025	0.201 ± 0.031	47.00R
Caudal	0.176 ± 0.020	0.192 ± 0.018	0.220 ± 0.030	0.248 ± 0.035	0.279 ± 0.041	58.62R
Liver						
Left lobe Anterior	0.209 ± 0.023	0.215 ± 0.020	0.232 ± 0.024	0.251 ± 0.020	0.285 ± 0.031	36.15R
Left lobe Posterior	0.160 ± 0.019	0.173 ± 0.014	0.189 ± 0.024	0.200 ± 0.031	0.234 ± 0.043	51.10R
Right lobe Anterior	0.160 ± 0.019	0.178 ± 0.014	0.195 ± 0.024	0.218 ± 0.031	0.242 ± 0.031	51.10R
Right lobe Posterior	0.200 ± 0.025	0.212 ± 0.025	0.230 ± 0.033	0.252 ± 0.040	0.281 ± 0.051	40.36R
Gills						
Left lobe	0.088 ± 0.011	0.092 ± 0.015	0.108 ± 0.012	0.118 ± 0.014	0.132 ± 0.017	50.15R
Right lobe	0.060 ± 0.013	0.068 ± 0.013	0.073 ± 0.010	0.084 ± 0.012	0.095 ± 0.009	58.06R

Values are mean ± SDM of 6 replicates. /Enzyme activity ug of Pi/mg protein at 37°C/. "F" test was performed. "F" is 0.05. Side script "F" and "R" indicates Fall and Rise respectively.

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EFFECTS OF HEAVY METALS ON THE CHEMOSENSITIVITY OF NEURONAL SOMATA OF *LYMNAEA STAGNALIS* L.

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Heavy metals are components in a number of chemical substances used both in agriculture and industry and potentially constitute an environmental problem. Most of the toxic heavy metals may affect the nervous system and cause neurological disorders (Nordberg 1980, Clarkson 1972). The understanding of the site and molecular mechanism of their action on nerve cells may greatly enhance our knowledge in order to prevent neural disturbances caused by heavy metal pollutants. On the one hand, lower animals can be used as a model system in clarifying this question. On the other hand, a number of reactions of aquatic animals caused by sublethal concentrations of heavy metals also occur as a result of alteration of the neural regulation, therefore revealing of the action of these substances at neuronal level can give an explanation for the evoked reactions and can be indicative of the toxic effect of heavy metals.

In behavioral responses the participation of the central nervous system is obvious. Although the primary target of the polluting substances should not be in all cases the nervous system, it is reasonable to suppose that nerve cells are under the direct influence of drugs present in the water and passing through the skin, alimentary canal, respiratory and circulatory systems to the brain.

Heavy metals may influence the nerve cell function either at the soma membrane or at interneuronal connections,

or both. Since one of the main functional properties of these sites is their specific response to naturally occurring transmitter substances /Gerschenfeld 1973/, the aim of the present investigations was to study the acute effect of Cd^{2+} and Hg^{2+} ions on the chemical sensitivity of the neurons of the giant pond snail, Lymnaea stagnalis L.

MATERIAL AND METHODS

The experiments were carried out on neurons of the isolated suboesophageal ganglionic ring of Lymnaea stagnalis L. /Gastropoda, Mollusca/ bathed in physiological saline /Carriker 1946/. The isolated ganglion complex of the Lymnaea brain was pinned to the Sylgard in a way that neurons of the dorsal surface became available. Some of these nerve cells have been identified earlier using retrograde $CoCl_2$ staining /S.-Rózsa and Salánki 1974/. The location of the central neurons used in the present experiments is shown in Fig.1.

The electrical activity of the neurons was recorded with the conventional microelectrophysiological method using KCl-filled glass-microelectrodes. The larger cells of the visceral and right parietal ganglia /sizes varied between 100-200 µm/ were selected for the experiments. The preparation was perfused permanently during control conditions with physiological saline, and afterwards with the saline containing heavy metals. For displaying and recording the action potentials four-channel Tektronix oscilloscope /R5103N/ and Gould--Brush recorder were used.

The specific response of the neurons to transmitter substances was studied under control conditions and following heavy metal treatment. Among heavy metals $CdCl_2$ and $HgCl_2$ were used, while sensitivity of the neurons was tested to the neurotransmitters acetylcholine /ACh/, 5-hydroxytryptamine /5HT/ and dopamine /DA/. Both the heavy netals and the neurotransmitters were used at 10^{-6} mol/l concentration.

Transmitter substances were applied either in drop application in the bath, or in a small quantity from a

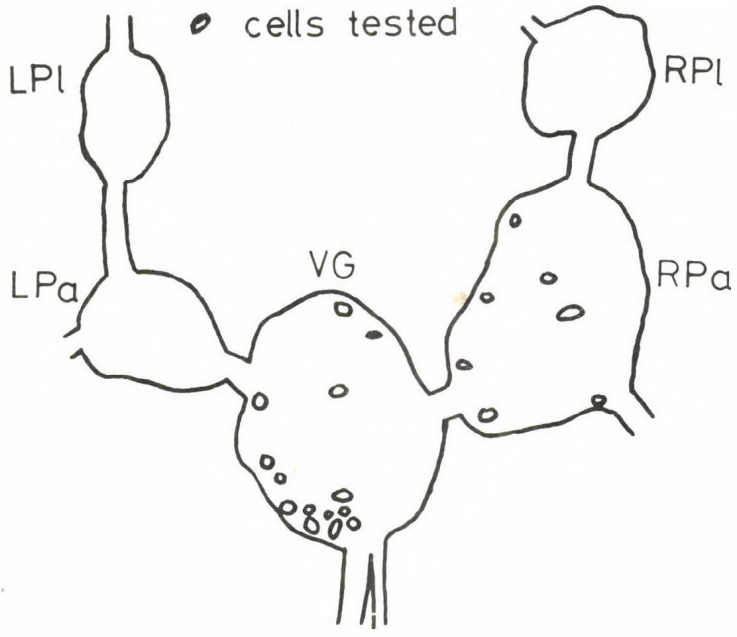

Fig. 1 - Schematic localization of the tested neurons in the central nervous system of Lymnaea stagnalis L.
VG - visceral ganglion, RPa - right parietal ganglion, LPa - left parietal ganglion, RPl - right pleural ganglion, LPl -left pleural ganglion.

micropipette directly to the soma of the investigated neuron. In the former case all three neurotransmitters were tested on the same neurons before and after heavy metal perfusion, however in the latter case on each neuron only one of the substances could be tested.

RESULTS

Under control conditions most of the investigated neurons responded to the application of ACh, 5HT or DA. Treatment of the ganglia with 10^{-6} M $CdCl_2$ or $HgCl_2$ solution usually did not cause any noticeable effect on the membrane potential or spike generation within 30 min. In most cases the response to transmitter substances did not change either, however, in about 20 per cent of the neurons slight or dramatic changes occurred in the chemical sensitivity. These results are to be demonstrated in the following.

1. Interaction of the cadmium ions with transmitter effects

The investigated Lymnaea neurons showed varying response to the ACh application depending on the membrane receptors. Its effect was excitatory, inhibitory or biphasic, similarly to that reported earlier /Vulfius et al 1967, Kiss 1973, Winlow and Benjamin 1976/. In Fig.2 the biphasic effect of ACh is demonstrated when the short increase in firing frequency of the neuron is followed by a weak inhibitory phase. However, following, $CdCl_2$ treatment the ACh effect was modulated and as a result an excitatory response of long duration appeared.

On another neuron the biphasic effect of ACh was much more expressed than in the previous case /Fig.3, upper/, but here, contrarily, the excitatory phase was eliminated under the influence of $CdCl_2$ and the inhibitory one prevailed /Fig.3, lower/. In this neuron bursting pattern was also formed following $CdCl_2$ treatment.

On a third neuron neither the excitatory nor the

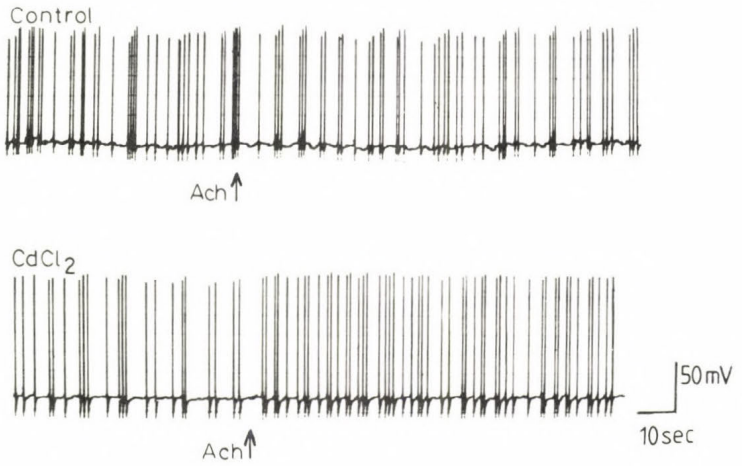

Fig. 2 - Turning the ACh inhibitory effect /10^{-6}M/ into excitatory following $CdCl_2$ /10^{-6}M/ treatment.

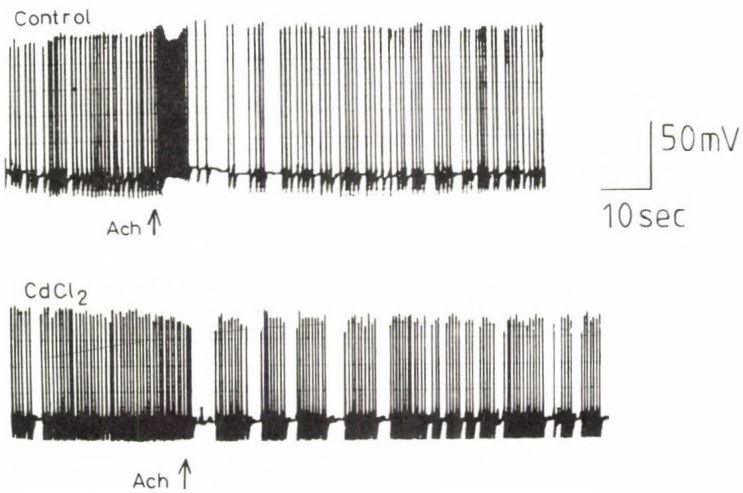

Fig.3 - Excitatory-inhibitory effect of ACh /10^{-6}mol/l/ turned to inhibitory after treatment with 10^{-6} mol/l $CdCl_2$.

inhibitory phases of ACh response were eliminated by $CdCl_2$ treatment, moreover both were enhanced /Fig.4A,B/. The same neuron showed no sensitivity to DA under control conditions but it was excited by DA following $CdCl_2$ treatment /Fig.4C,D/.

5HT caused inhibitory or excitatory responses on various Lymnaea neurons and both effects were eliminated by $CdCl_2$ treatment /Figs 5 and 6/.

The response to dopamine changed also differently in various neurons. Figure 7 demonstrated that the inhibitory effect of DA was not only eliminated but turned into an excitatory one following $CdCl_2$ treatment. A reverse of this response occurred in another case, when instead of an excitatory effect inhibitory effect appeared after $CdCl_2$ treatment /Fig.8/.

2. Modulation of transmitter effects by $HgCl_2$

$HgCl_2$ proved to be also an effective modulator of transmitter effects. In one neurone $HgCl_2$ treatment turned the inhibitory response of ACh to excitatory or biphasic /Fig.9/. In this neuron at the beginning of $HgCl_2$ application the firing of the neuron was inhibited, however, the sensitivity to ACh remained and manifested as a burst of high-frequency discharges /Fig.9, middle line/. The excitatory response caused by ACh following $HgCl_2$ treatment showed no desensitization, moreover, some sensitization could be observed after 10 min application of $HgCl_2$ when firing of the neuron reappeared /Fig.9/.

The alteration of the type of transmitter effect was rather specific following $HgCl_2$ treatment. As can be seen in Fig.10, the biphasic excitatory-inhibitory effect of ACh turned to inhibitory, while the inhibitory effect of 5HT became excitatory following $HgCl_2$ treatment in the same Lymnaea neuron /Fig.10/. The inhibitory DA response was also modified, as under the influence of $HgCl_2$, when the activity of the neuron was depressed, DA caused a single action potential /Fig.11/ showing the sensitization of the membrane DA receptors.

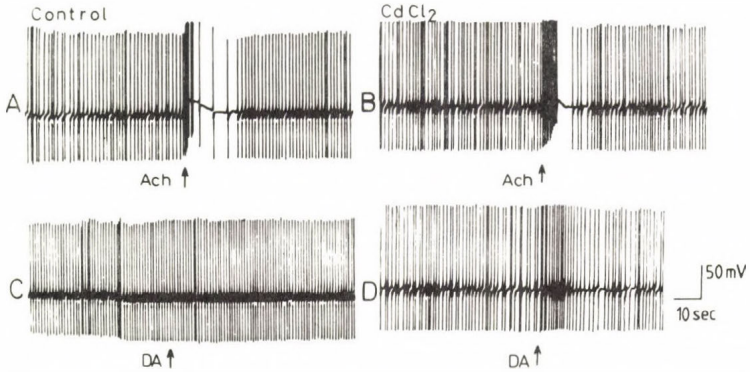

Fig. 4 - Excitatory-inhibitory effect of ACh /10^{-6} mol/l/ was enhanced /A and B/ and non-sensitive neuron became stimulated by DA /10^{-6} mol/l/ /C and D/ after treatment with 10^{-6} mol/l $CdCl_2$

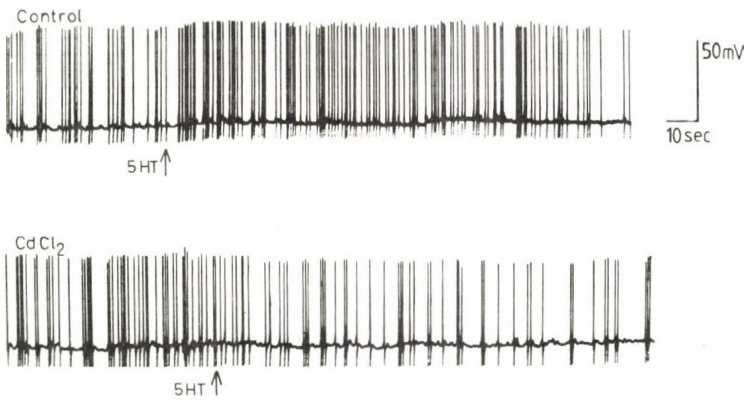

Fig. 5 - Excitatory effect of 5HT turned into inhibitory following $CdCl_2$ treatment.

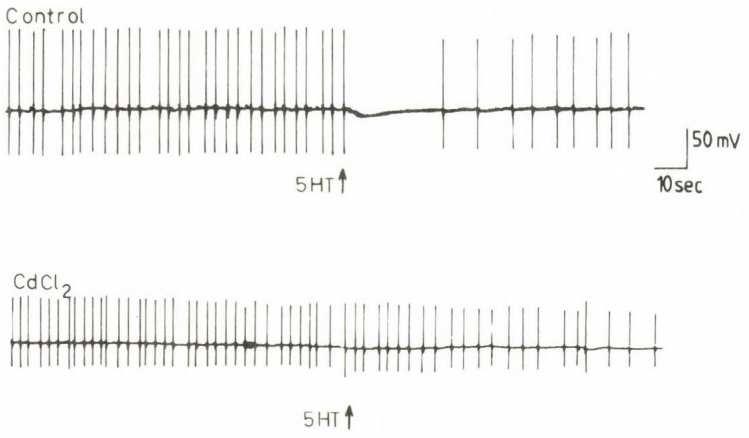

Fig. 6 - Inhibitory effect of 5HT /10^{-6} mol/l/ became eliminated after treatment with 10^{-6} mol/l $CdCl_2$.

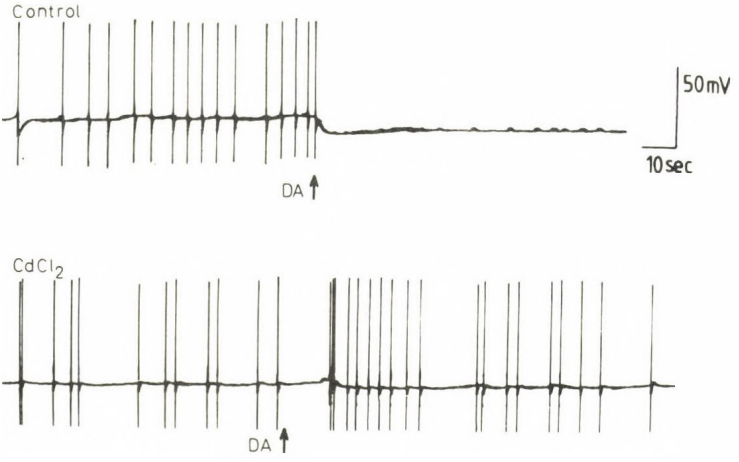

Fig. 7 - Inhibitory effect of DA /10^{-6} mol/l/ turned to excitatory after treatment with 10^{-6} mol/l $CdCl_2$.

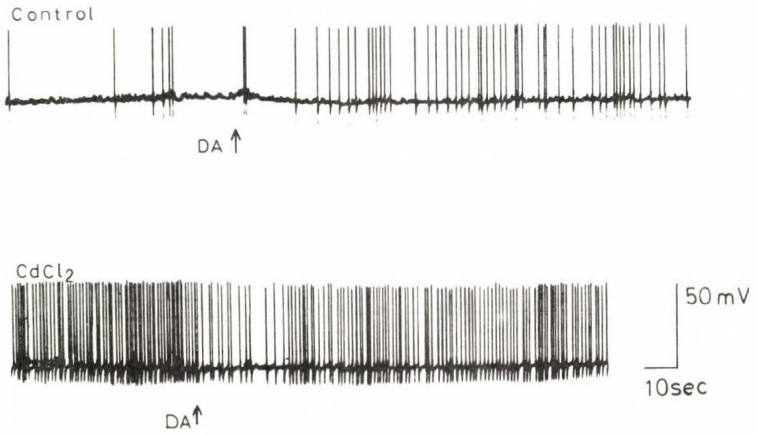

Fig. 8 - The excitatory effect of DA became inhibitory after $CdCl_2$ treatment.

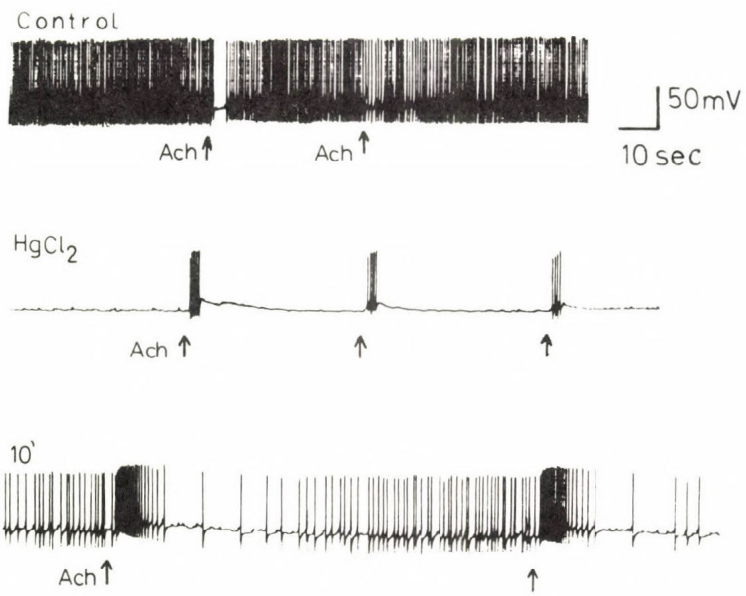

Fig. 9 - Inhibitory effect of ACh /10^{-6} mol/l/ turned to excitation after treatment with 10^{-6} mol/l $HgCl_2$.

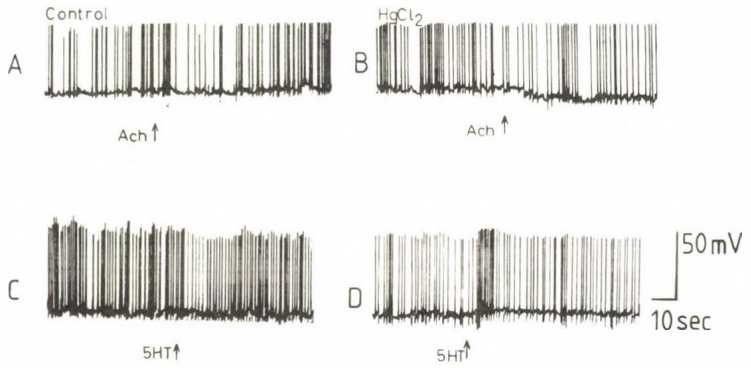

Fig. 10 - Excitatory effect of ACh /10^{-6} mol/l/ turned to inhibitory /A and B/, while inhibitory effect of 5HT turned to excitatory /C and D/ under the effect of 10^{-6} mol/l $HgCl_2$.

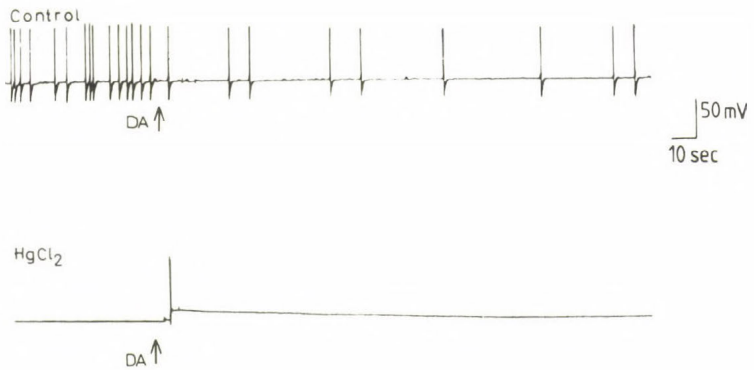

Fig. 11 - Instead of the inhibitory effect of DA /10^{-6} mol/l/ depolarization occurred after treatment with 10^{-6} mol/l $HgCl_2$, when spiking of the neuron was inhibited.

DISCUSSION

The results suggest that heavy metals may cause a very profound modulation on the elements of the neural circuitry responsible for the regulation of the animal's behavior. The observed effects can be explained by blocking or modifying cholinergic, dopaminergic or serotonergic receptor structures as well as by influencing ionic channels located at the soma membrane.

It is well known that the active groups of the receptors for many neurotransmitters can be altered by heavy metals /Clarkson 1972/. The $CdCl_2$ can also interfere with regulatory processes controlled by Ca^{2+} ions and disturb in this way neurotransmitter release and uptake as well as electrophysiological properties of the nerve membrane /Karlin and Bartels 1966, Stadel and Lefkowitz 1979, Kostyuk 1980/. The mercury ions have high affinity to sulfhydryl /-SH/ and disulfide /S-S/ groups and it is generally accepted that their toxicological effect is based on the mercury-sulfur interactions. The transmitter related effect of mercury ions can be a consequence of the interference of mercury ions with ionic mechanisms of neurotransmission, enzyme regulations, transport processes, protein phosphorylation in the membrane and with many other vital events at extra-, and intracellular level. The involvement of sulfhydryl groups in the maintenance of the structure and function of many membrane bound receptors has been observed /Karlin and Bartels 1966, Stadel and Lefkovitz 1979/. The SH-groups are uniquely involved in the formation of high-affinity agonist-receptor complexes.

Our results are consistent with the above described mode of heavy metal effects. Although only about twenty per cent of the investigated neurons of Lymnaea was affected by cadmium and mercury ions, these neurons may be responsible for the vital processes of the animal. The facts that the pattern of firing of the affected neurons can basically change, the effect of the neurotransmitters can be eliminated or it can turn into

the opposite, moreover, neurons can be sensitized to neurotransmitters, suggest that under the effect of Cd or Hg the whole regulatory process can be altered which will have its consequences in the animal's behavior.

At the same time, the physiological effect of the investigated heavy metals can be regarded as specific on the nerve membrane of Lymnaea. The transmitter related effects of heavy metals can be connected to the alteration of ionic mechanisms of neurotransmission, which can precede neuropathological damages. However, the modification of receptors binding ACh, 5HT or DA can also be involved into heavy metal effects, as well as, modification of intracellular processes responsible for the regulation of membrane permeability /Vadász and Salánki 1982, Salánki et al 1983/.

The results showing very definite alterations in the chemosensitivity of the neurons suggest that molluscan nerve cells can be useful tools for predicting early, sublethal effects not only of heavy metals, but also of other anthropogenic pollutants, affecting animal behavior.

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DISCUSSION

WEIS, P: Some molluscan nervous systems have been analysed to find out which neurons have which functions. Is this so for Lymnaea, and if so, what is the function of the neurons which you have manipulated?

S.-RÓZSA, K: Similarly to other molluscan species the number of central neurons has also been identified in Lymnaea stagnalis both morphologically and physiologically. The neurons in question are involved mainly into the regulation of visceral functions.

Symposia Biologica Hungarica 29 (1985)

HEAVY METAL POLLUTION INFLUENCES SEROTONIN LEVEL AND DOPAMINE-STIMULATED ADENYLATE CYCLASE ACTIVITY IN THE CNS OF MOLLUSCS

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INTRODUCTION

It is well known that filtration and feeding activity of mussels depend on the periodic activity of the adductors and the ciliary activity of the gills. At the same time heavy metals are known to reduce the filtration activity of mussels /Salánki 1966, Salánki and Lukacsovics 1967/.

Monoamines are present in the nervous tissues of a variety of invertebrates /Von Euler 1961, Sweeney 1963, Dahl et al. 1966, Welsh 1972, Osborne 1977/ including the bivalve molluscs Anodonta cygnea /Hiripi 1968, 1972/ and Mytilus edulis /Stefano and Catapane 1977a,b/. In our earlier work it was demonstrated that monoaminergic mechanisms are involved in the regulation of both the periodic activity of Anodonta cygnea /Salánki et al. 1974, Hiripi 1977/ and the ciliary activity of Mytilus edulis /Catapane et al. 1978, 1979/.

Certain neurotransmitters may exert some of their effects in nervous tissue by stimulating the formation of cyclic AMP. The nervous tissue of various vertebrates and invertebrates contains a dopamine sensitive adenylate cyclase /Kebabian and Greengard 1971, Cedar et al. 1972, Kebabian and Saavedra 1976, Treistan and Levitan 1976, Osborne 1977/. Mytilus edulis pedal ganglia also have been shown to contain dopamine-stimulated adenylate cyclase /Stefano et al. 1981/. The same type of cyclase also exists in Mytilus edulis peripheral tissues /Malanga et al. 1980/.

As monoamines regulate the activity of the animals and the cAMP system is involved in some effect of the monoamines we wondered whether heavy metals affect directly these system or not. In Anodonta cygnea L. we investigated the in vivo effect of cadmium and lead on the serotonin content of the ganglia. In Mytilus edulis we studied the in vitro effect of lead, mercuric, cupric, zinc, ferric and nickelous ions on the dopamine stimulated adenylate cyclase activity.

Materials and Methods

Specimens of adult Anodonta cygnea L. were collected from fish ponds. Before the experiments the animals were kept in aquarium supplied with Balaton-water. The flow rate of the water was 20 l/h. In the experiments Cd^{2+} was added as $CdSO_4$, Pb^{2+} as $PbCl_2$. During the experimental period metal solution was pumped into the aquarium instead of natural Balaton-water. Stock solution was prepared and added to the experimental tank after dilution with the inflow of Balaton-water. The flow rate was also 20 l/h, and the concentrations of both metal solutions were 12.5 µg/l. The temperature of the water varied between 16-20 ^{o}C.

The serotonin content was measured separately in the cerebral, pedal and visceral ganglia using high pressure liquid chromatography with fluorescence detection. The ganglia were homogenized in 100 µl 0.1 N perchloric acid. The homogenates were centrifuged at 30.000 rpm at 4^{o}C for 20 min and 50 µl of the supernatant was injected into the LC. Waters liquid chromatograph with a U6K injector, Waters µBondapack C_{18} reserve phase column and Aminco Bowman fluorimeter as a fluorescence detector were used. The mobile phase was 0.05 M sodium acetate pH 4.7 containing 5 % methanol. The flow rate was 1.5 ml/min. The uncorrected excitation and emission wavelenghts were 295 and 330 mµ, respectively.

Specimens of subtidal Mytilus edulis were collected from Long Island Sound at Northport, New York, and used within 2 hrs of collection. The pedal ganglia of freshly collected

Mytilus edulis were dissected on ice, and the pooled pedal ganglia from 240 animals /approximately 0.2 mg of protein/ganglion/ were gently homogenized by hand with a ground glass homogenizer in 15 ml of Tris-malate buffer /2mM, pH 7.4/ containing EGTA /0.8 mM/. The homogenate was kept at 4°C. Adenylate cyclase activity was measured according to the method of Walczak et al /1979/ with modifications. The assay system contained 80 mM Tris-malate buffer /pH 7.4/, 5 mM theophylline /to inhibit phosphodiesterase activity/, 2 mM $MgSO_4$, 0.5 mM ATP, 0.2 mM EGTA, 50 µl of homogenate and the test agents /metals/ as indicated in a total volume of 100 µl. The mixture was incubated for 4 min at 23°C in a shaking water bath. The reaction was started by adding ATP to the system and was terminated by placing the assay tubes in a boiling water bath for 2.5 min. After termination, the incubation mixture was centrifuged at a low speed to remove particulate material, and 50 µl aliquots of the resultant supernatant were assayed for cyclic AMP utilizing the Amersham cyclic AMP kit. Quadruplicate incubations for each test condition were run for each experiment. Additionally, duplicate cyclic AMP determinations were made for each incubation. Recoveries were calculated by the addition of known amounts of labelled cyclic AMP. The results were corrected for recoveries and expressed as mean values ± SEM. Protein concentration determinations were made according to the method of Lowry et al. /1951/. The Student t-test was used to determine the statistical significance of the data. The IC_{50} values, at the concentration of heavy metal required to produce half-maximal response was determined from a log-probit plot of the per cent of increase in basal cyclic AMP level versus concentration of the added metal.

Results

Effect of Cd and Pb on the serotonin content of the ganglia

The in vivo treatment of the animals with Cd^{2+} influenced moderately the serotonin content of the ganglia /Fig.1/.

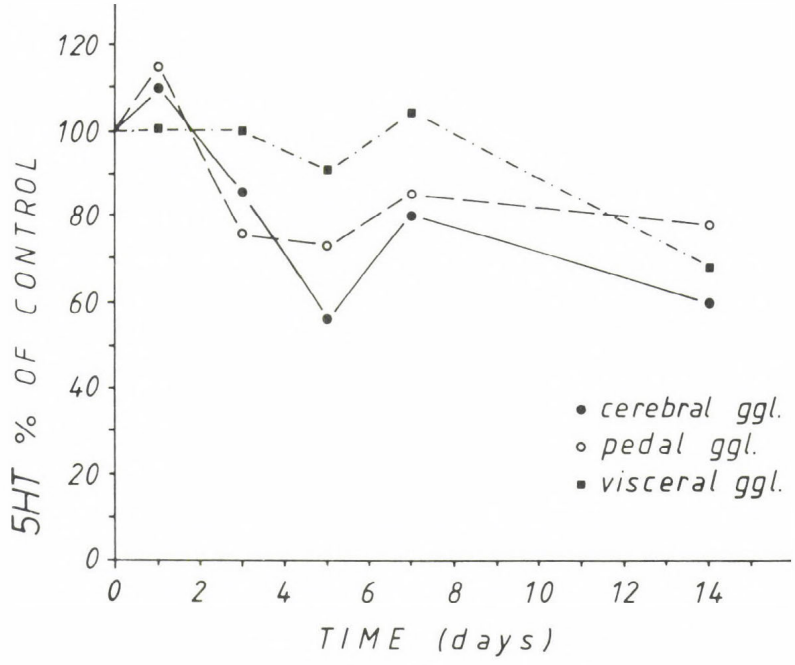

Fig.1 - Alteration of serotonin content in the ganglia of Anodonta cygnea treated with Cd^{2+}.

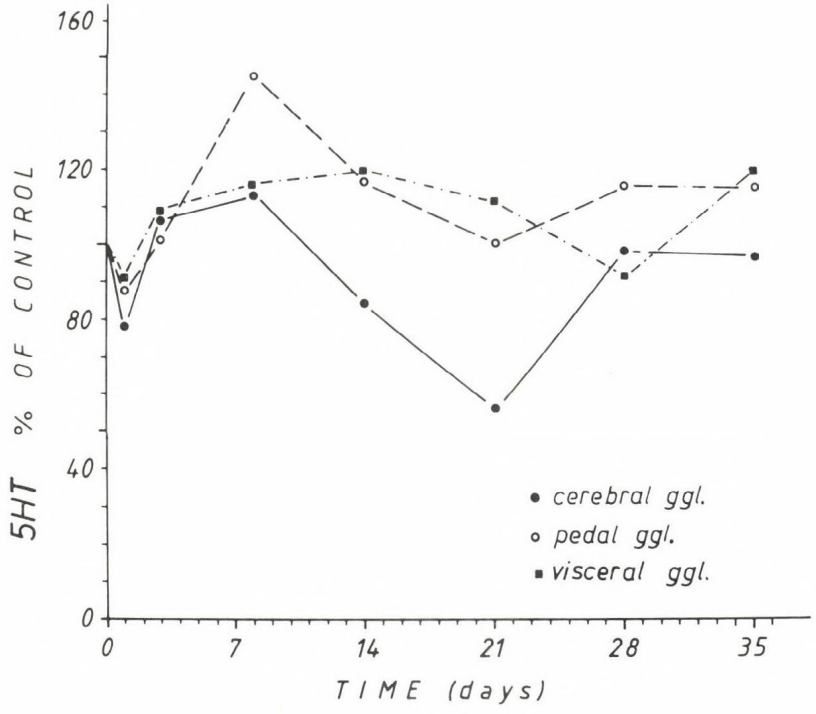

Fig.2 - Alteration of serotonin content in the ganglia of Anodonta cygnea treated with Pb^{2+}.

In the cerebral and pedal ganglia after a small transient increase measured on the first day the serotonin content started to decrease. On the 5th day the decrease of the serotonin content in the cerebral and pedal ganglia was 25 and 40 per cent, respectively. In the visceral ganglia there was no change in the serotonin content during the first week after the treatment. By the end of the second week, however, the serotonin content was decreased also in these ganglia by 30 per cent.

Exposure of the animals to Pb^{2+} influenced also the ganglionic serotonin content /Fig.2/. On the first day after treatment the serotonin content showed a small, transient decrease in all ganglia. One week after the treatment the serotonin concentration of the pedal ganglia increased by 45 per cent, which returned to the control level by the end of the third week of the exposure. In the cerebral ganglia the serotonin content decreased during the 2nd and 3rd weeks then returned to the control value in the 4th week. There were no significant changes in the serotonin content of the visceral ganglia during the investigated period.

Effect of heavy metals on the dopamine stimulated adenylate cyclase activity

The addition of 100 μM of dopamine to the incubation medium /in vitro/ increased the cyclic AMP content of the pedal ganglion homogenates. The basal level of 14.72 ± 1.08 pmol/mg protein increased by 250 % at 4 min as previously noted /Stefano et al. 1981/. Lead, mercuric, cupric and zinc chloride were potent inhibitors of dopamine-stimulated adenylate cyclase activity /Table 1/. Both ferric and nickelous chloride were less potent. It is important to note that at concentrations as low as 0.4 μM of lead and mercuric chloride the dopamine--stimulated adenylate cyclase enzyme was inhibited to a significant extent.

Table 1 - Effects of heavy metals on dopamine-stimulated adenylate cyclase activity

Metal	Dopamine-adenylate cyclase inhibition /IC_{50}/ μM
Lead chloride	3.1
Mercuric chloride	3.3
Cupric chloride	3.9
Zinc chloride	4.1
Ferric chloride	87.4
Nickelous chloride	97.8

IC_{50} - values were determined from dose-response curves consisting of six points within the concentration range of 0.01-1.000 μM. Each point represented the mean of three replicate samples assayed for cyclic AMP levels according to Stefano et al. 1982.

Discussion

Heavy metals are accumulated into different living organism, although most of them are elements biological action of which is not involved in any essential cellular function. However, some of these elements have a toxic effect on many organ systems and the central nervous system may be the most sensitive. The neurotoxic effect of the heavy metals as lead, mercury, cadmium, copper were studied mainly in the vertebrates. It was found that their effect is rather specific for different neurotransmitter pathways within the central and peripheral nervous system. There is evidence that lead

increases in vivo the catecholaminergic, primarily dopaminergic activity /Silbergeld 1982/. However, both increased and decreased levels of serotonin have been reported in the CNS of rat and mice under the effect of lead by Hrdina et al. /1980/. The results presented here show that lead and especially cadmium influence the ganglionic serotonin content of Anodonta cygnea. Exposure of the animal to cadmium decreased the serotonin content in all the ganglia but with different times. Lead caused a transient increase of serotonin in the pedal ganglia, and a considerable decrease in the cerebral ganglia. Lead, mercuric, cupric and zinc ions could inhibit the dopamine stimulated adenylate cyclase in the pedal ganglia of Mytilus. These heavy metals have a potency to decrease the activity of the fresh water mussel /Salánki and Varanka 1976, V.-Balogh and Salánki 1984/. Using different pharmaca which influenced both the serotonin level and the periodic activity we have demonstrated that the decreased serotonin level was associated with decreased activity of the animal /Hiripi 1977/. Various ganglia play different roles in the regulation of the mussel's activity /Salánki et al. 1968/ and there are also differences in the serotonin concentration of the cerebral, visceral and pedal ganglia /Hiripi 1968/. The divergent effect of heavy metals on the serotonin level of various ganglia of Anodonta can be connected with these peculiarities. Most probably cadmium inhibits the serotonin synthesis causing a lowering of the serotonin level in the ganglia, which results in the decreasing of the activity. Other heavy metals may act similarly on the monoaminergic mechanisms of the ganglia.

The question where heavy metals may affect the monoaminergic system or its regulatory function in mussels cannot be answered yet.

Intracellular events as the synthesis, the breakdown, the liberation, the reuptake, the binding to the receptor can be all targets, as well as the interaction with the second messenger system. Relatively little work has been done on the influence of heavy metals on neurotransmitter/neuromodulator mechanisms in neural tissues. This is especially

true of the receptor interaction of a signal molecule with the intracellular second messenger system. In 1976, Nathansen and Bloom reported that at μM concentrations heavy metals could inhibit a norepinephrine-sensitive adenylate cyclase mechanism in rat submandibular salivary gland. The present study also finds these metals effective in inhibiting dopamine-stimulated adenylate cyclase activity within the same concentration range reported in the mammalian system. The similarities between dopamine-stimulated adenylate cyclase of mammals and of other invertebrates /Osborne 1977/ thus appear also to exist for Mytilus edulis /Stefano et al. 1981/.

The present results suggest that the investigated heavy metals may influence both the synthesis of the serotonin and the response of the second messenger system to monaomines. At the same time Anodonta cygnea, Mytilus edulis and other invertebrates may represent a new area for the investigation of heavy metal toxicity. Indeed, given the vital regulatory activities associated with the nervous system, its responsive mechanisms to environmental assualts may be critical to our understanding of the mechanism of action of potentially harmful agents.

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EFFECT OF Cu ON SOME BIOCHEMICAL PARAMETERS OF FISHES

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INTRODUCTION

During the last two decades, as a result of industrial development in Hungary, the concentration of copper in rivers and lakes has increased, causing a rise of copper level in fishes (Salánki et al. 1982). Copper has been found to have physiological and biochemical effects on fishes (Weis and Weis 1977; Labat et al., 1977; Waiwood and Beamish, 1978; Dixon and Sprague, 1981; Nemcsók and Boross, 1981; 1982; Finlayson and Verrue, 1982; Rojik et al., 1983; Nemcsók et al., 1984).

Many authors demonstrated the adverse effect of anthropogenic agents by the determination of ASAT (aspartate aminotransferase, EC 2.6.1.1.); ALAT (alanine aminotransferase, EC 2.6.1.2.); LDH (lactate dehydrogenase, EC 1.1.1.27) and AChE (acetylcholinesterase, EC 3.1.1.7) (Kristoffersson et al.,1974; McKim et al., 1970; Reichenbach-Klinke, 1972). Moreover, environmental pollution may produce stress in fishes with changes in plasma lactate and glucose level (Wedemeyer, 1970).

The aim of our work was to carry out studies regarding the effect of $CuSO_4$ on the above mentioned biochemical parameters in fish species with different nutritional habits.

MATERIALS AND METHODS

Common carp (Cyprinus carpio L.), silver carp (Hypophthalmichthys molitrix V.) and European wels (Silurus glanis L.) specimens of 350-450 g were obtained from Fisheries Research

Institute in Szarvas, and held for a minimum of 7 days before experimentation in a 100 litre aquarium (5 fishes per aquarium) at a temperature of 20 $\pm$1 ^{o}C. The length of exposure to $CuSO_4$ at high concentration (10 ppm) was 2 hours at low concentration (5 ppm) for 24, 48, 96 hours and for 1 or 2 weeks. $CuSO_4$ was added to the given concentrations into the aquarium water.

For the determination of ASAT and ALAT and LDH activities Boehringer kits were used. AChE activity was measured with the method of Ellman et al. (1961). Measurements performed using a colorimeter (SPECTROMOM 198).

Blood glucose was determined by the glucose oxidase-peroxidase method.

The determination of proteolytic enzyme activity was carried out by the method of Anson (1938).

RESULTS AND DISCUSSION

After the 2-hour $CuSO_4$ treatment (10 ppm), the biochemical parameters have changed significantly in carps compared to the controls (Figs 1,2). The ASAT activity increased by about 30% and the ALAT activity was three times as high as in the controls. The most significant changes were observed in silver carp. The ASAT activity duplicated, the ALAT and the LDH activities were 2.5 and 4 times higher, the blood glucose level duplicated. $CuSO_4$ - similar to the carp and silver carp - enhanced serum ASAT, ALAT, LDH activities and blood glucose level were found in the wels. However, $CuSO_4$ was less toxic to wels than to the other two species.

When the exposure time was maximum 2 weeks (at 5 ppm $CuSO_4$ concentration) the ASAT and ALAT activity was generally enhanced in the serum as well. At the end of the second week the ASAT activity was 6 times more intensive than in the controls.

During the long-term treatment with $CuSO_4$ the serum LDH activity and blood sugar level increased as well. At the end of the second week the serum LDH activity was 15 times higher than in the controls. Blood sugar level reached its maximum after the treatment of the first week, when it was 3 times higher compared to the controls (Fig.4).

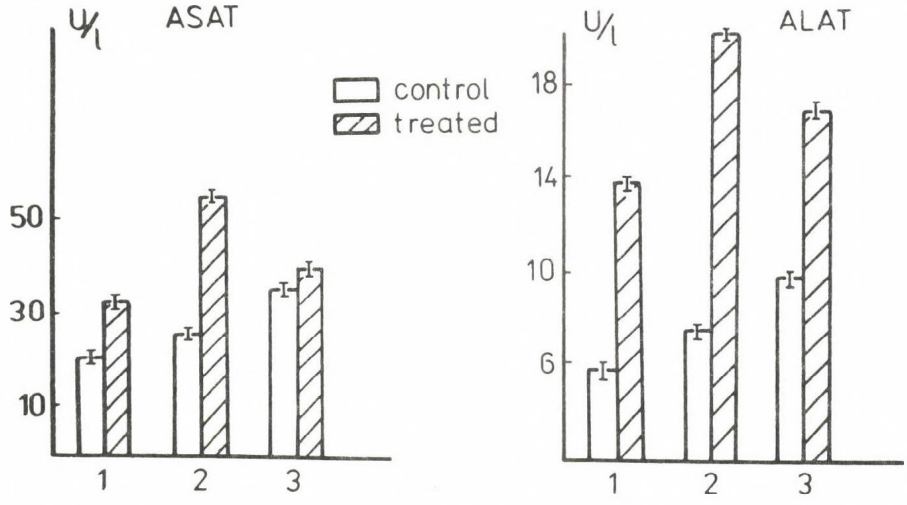


Figure 1.: The effect of 10 ppm $CuSO_4$ on blood serum ASAT and ALAT activity of carp (1), silver carp (2) and wels (3). Exposure time 2 hours. The values represent averages for 8-12 specimens ($\pm$ S.D.)

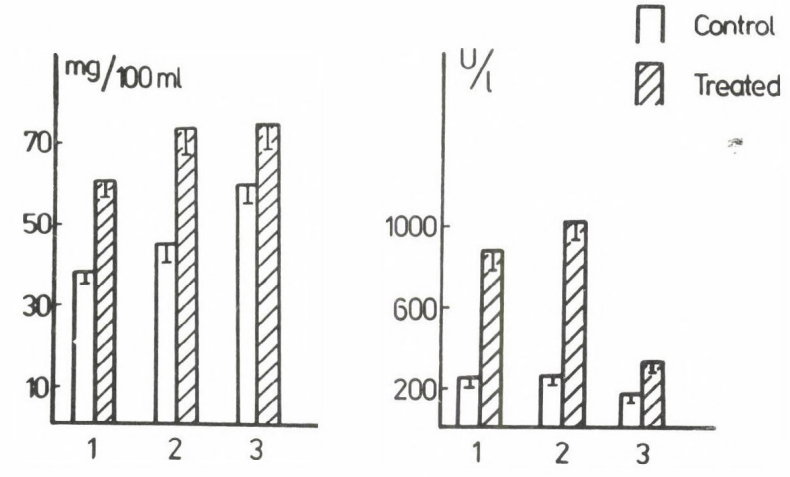
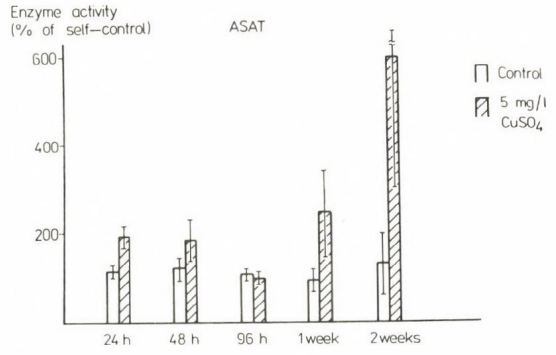


Figure 2.: The effect of 10 ppm $CuSO_4$ on blood sugar level and LDH activity of carp (1), silver carp (2) and wels (3). Exposure time 2 hours. The values represent averages for 8-12 specimens ($\pm$ S.D.).

These results clearly show that the toxicity of $CuSO_4$ affected the three investigated fish species differently. Bell (1968) studied the effect of hepatic poisons on ASAT activity,

using bromobenzene and carbon tetrachloride in high doses. He found that in the treated fishes the ASAT was significantly elevated. Even in fish with diseased kidneys he demonstrated a significant increase in plasma ASAT activity as compared to the controls. Kristoffersson et al. (1984) reported that 5 ppm phenol increased the ASAT and ALAT activity in the pike (Esox lucius L.) In our experiment, $CuSO_4$ enhanced ASAT and ALAT activities in all three investigated species, but the degree of changes was different. The highest ASAT and ALAT activities were measured in silver carp, reflecting a serious damage of tissues. The increase in ASAT and ALAT activities in the carp was less significant. The slightly increased ASAT and ALAT activities of wels as compared to the control refers to the fact that the tissue damage was not so significant. This slight difference between the treated and control animals might be due to the highest ASAT and ALAT activities in the control wels. However, Onishi and Murayama (1970) demonstrated that the hepatic ASAT activity does not differ much according to species. The elevated ASAT and ALAT activities were presumably due to damage of the liver, but other organs may also have been damaged (kidney and/or gill) (Rojik et al., 1983). Reichenbach-Klinke (1972) reported the damage of the gill after $CuSO_4$ treatment. Schreck et al. (1978) observed that kidneys of Cu-exposed fish had glomerular atrophy and epithelial necrosis of the gills.

Blood glucose appeared to be a sensitive, reliable indicator of environmental stress in fishes. On the basis of our results it is clear that $CuSO_4$ - as it is shown by the elevated blood glucose level - acted as stressor on fishes. The order of magnitude of the stress effect is silver carp>carp>wels. The increased LDH activity showed the same order, presenting the metabolic changes in stressed fishes: the catabolism of glucose moved towards the lactic acid which is very dangerous and toxic to fishes (Leviasted et al., 1957; Nakono and Tomlinson 1967). The enhanced LDH activity in carp and silver carp could be due to the enhanced swimming activity and, contrary to $CuSO_4$, intoxicated wels which were resting on the bottom of the aquaria. So the enhanced LDH activity of carp and silver carp could be a



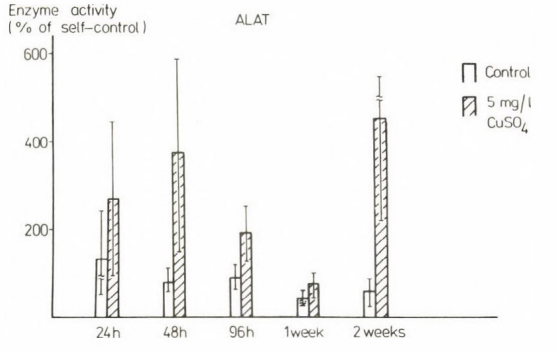


Figure 3.: The effect of 5 ppm $CuSO_4$ on blood serum ASAT and ALAT activity of carp. Exposure time 24, 48, 96 hours and 1, 2 weeks. The values are expressed in percentage of self controls representing 3-6 specimens ($\pm$ S.D.).

consequence of increased swimming activity.
Our results showed that wels tolerated the metal pollution well, while carp and silver carp were very sensitive to the mentioned environmental stressors.

The differences in the damaging effect of $CuSO_4$ regarding the three investigated fish species might be due to the different rates of their operculum movement and the altered microsomal enzyme activities that might metabolize the toxic metals as well (Simon et al., 1983; 1984).

Copper sulphate inhibited in vitro the AChE activity the most in the brain and the least in the heart. The greatest in-

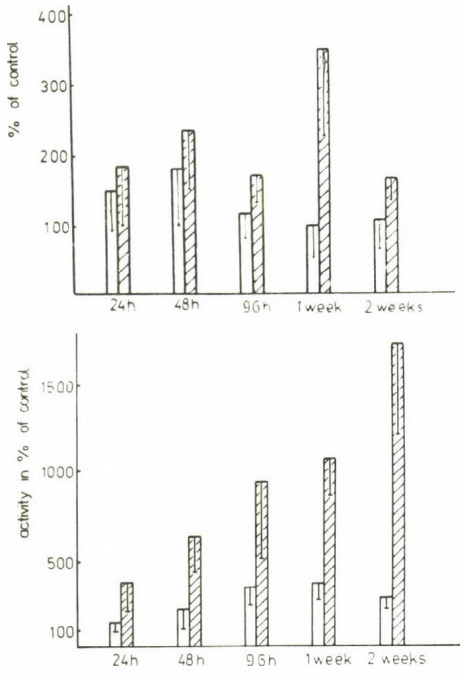


Figure 4.: The effect of 5 ppm $CuSO_4$ on blood sugar level and LDH activity of carp. Exposure time 24, 48, 96 hours and 1, 2 weeks. The values are expressed in percentage of self controls representing 3-6 specimens ($\pm$ S.D.).

hibition in vivo was observed in the muscles and the slightest in the brain (Fig. 5) which presumably, can be attributed to the presence of a blood-brain barrier. Copper sulphate markedly inhibited the enzyme activity in serum and brain in vitro. During in vitro exposures the 50% decrease of the AChE enzyme activity in the brain occurred at lower concentration as compared to the other organs.

By comparing IC_{50} values (Table 1) (concentrations producing 50% inhibition) obtained in vivo and in vitro, respectively, it may be inferred that copper sulphate concentration in the serum of in vivo exposure may be lower than in aquarium water, i.e. copper sulphate presumably either does not accumulate in the blood and/or it is rapidly removed and accumulated in other organs. It is more striking in the case of the brain, where the IC_{50} value measured in vivo is 30-fold of that obtained in vitro

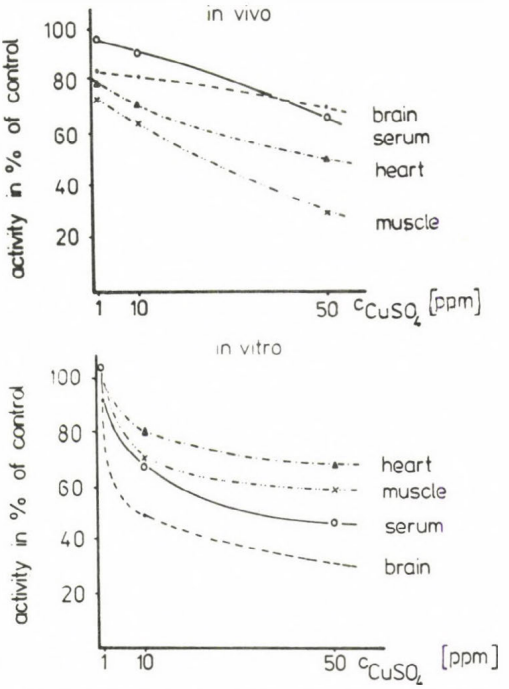


Figure 5.: Effect of $CuSO_4$ in vivo and in vitro on acetylcholinesterase activity in the serum, brain, heart and skeletal muscles of the common carp. The calues expressed in percentage of the controls are averages of measurements from 3-5 individuals.

which indicates that under in vivo conditions less copper sulphate penetrates into the brain than into other organs. Values of IC_{50} measured in muscles and heart in vivo were lower than those obtained in vitro, which indicates that copper sulphate may have accumulated in these organs to a larger extent. Support for these differences in accumulation has been obtained in our laboratory using radioactive isotopes of Cu in carp (Nemcsók, 1982 unpubl. data). The long time effect of $CuSO_4$ (5 ppm concentration and 2 weeks' treatment) on the AChE activity showed that inhibition could be observed on the first day only after the treatment (Fig.6).

Applying Lineweaver-Burks's approach, we found that copper sulphate in serum induced combined inhibition (Fig.7) because of the increased K_m constant and the decreased V_{max} values obtained.

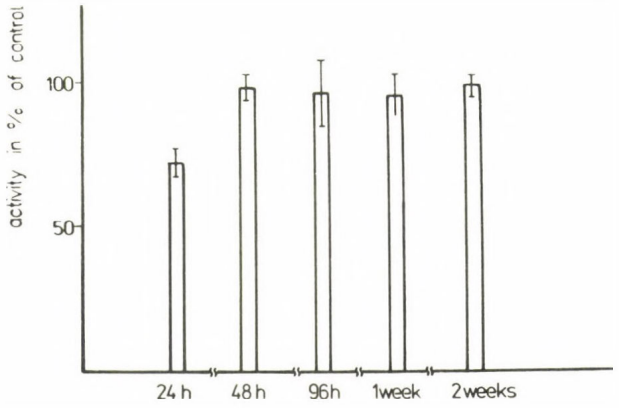


Figure 6.: The effect of 5 $CuSO_4$ on serum acetylcholinesterase activity of carp. Exposure time 24, 48, 96 hours and 1, 2 weeks. The values are expressed in percentage of the self controls, representing 3-6 specimens ($\pm$ S.D.).

TABLE 1.

$CuSO_4$ concentration values inducing 50% acetylcholinesterase inhibition in serum, brain, heart and skeletal muscles of common carp.

Organ	CI_{50} (M) in vitro	in vivo
Serum	$2.65 \pm 0.21 \times 10^{-4}$	$7.19 \pm 0.64 \times 10^{-4}$
Brain	$6.45 \pm 0.53 \times 10^{-5}$	$1.85 \pm 0.15 \times 10^{-3}$
Heart	$2.64 \pm 0.19 \times 10^{-3}$	$3.61 \pm 0.32 \times 10^{-4}$
Muscles	$1.04 \pm 0.09 \times 10^{-3}$	$1.54 \pm 0.13 \times 10^{-4}$

During the application of copper sulphate as a fungicide, concentrations significantly increased in the soil and copper could get into rivers and damage algae and fish (Cremlyn, 1978) through inhibition of AChE in the organisms. The copper ions are expected to bind to the thiol groups of the enzyme, but AChE is not sensitive to other thiol reacting groups (Silver, 1974).

According to Olson and Christensen (1980), AChE activity measured in the muscles of fathead minnow, Pimaphales promelas,

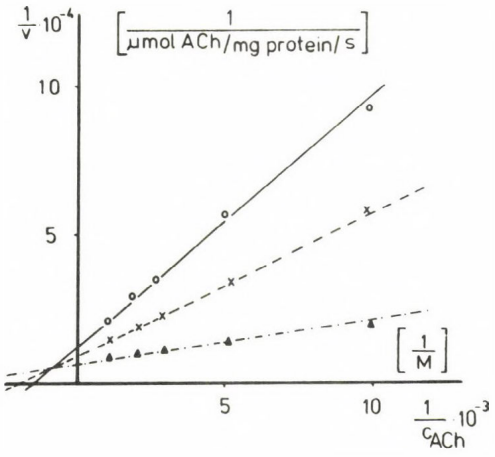


Figure 7.: Kinetic studies of $CuSO_4$ inhibition on serum acetylcholinesterase activity of carp according to the method of Lineweaver-Burk.

decreased to its half under in vivo exposures to 1.6×10^{-4} M copper ion concentration. Abou and Menzel (1967) found that AChE activity in the brain homogenate of Cymatogaster aggregata decreased by 20% upon exposure to 1.25×10^{-4} M copper sulphate in the water. This value is different from those we measured in vivo and in vitro, and may be attributed to the differences in sensitivity in the investigated fish species.

From our results we conclude that $CuSO_4$ markedly inhibited the AChE activity in vitally important organs of fish both in vivo and in vitro. Therefore inhibition of this activity may be used to assess exposure of fish to these herbicides in the natural environment.

$CuSO_4$ decreased the proteolytic enzyme activity in all investigated fishes already at 1 ppm.

The most significant decrease can be seen in wels (25%) and in carp (15%). There was only a slight change in silver carp (5%).

At 10 ppm $CuSO_4$ there was a remarkable decrease also in wels. However, at this concentration the proteolytic enzyme activity markedly decreased (50%) in carp and silver carp as well (Fig.8).

Reichenbach-Klinke (1972) reported 20-35% decrease of proteolytic enzyme activity in trout after 0.1-0.5 ppm $CuSO_4$. In our experiments such changes could be registered at higher (10 ppm) concentration only.

The difference in these data may be due to the sensitivity of different fish species and the different exposure to $CuSO_4$ pollution.

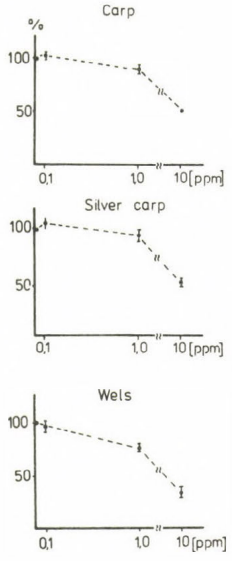


Figure 8.: The effect of 0.1, 1.0 and 10 ppm $CuSO_4$ on the proteolytic enzyme activity of carp, silver carp and wels. The values are the average of 3-9 fishes and are expressed in percentage of the controls. Exposure time 2 hours.

We have found that the Cu^{2+} reduced the proteolytic activity of these fish species. Though it is known that the Cu^{2+} ions accelerate the oxidation of SH-groups of proteins in our cases the inhibiting effect of this metal ion may be taken as a secondary effect followed by the changes in metabolic processes after Cu^{2+} treatment of fishes rather than a direct action on the centre of these enzymes, because as it was shown earlier (Jonás et al., 1980) these enzymes were not SH-type proteases.

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DISCUSSION

LORCH, D: Are the effects described for copper specific or do other heavy metals have similar effects on the enzyme level?

NEMCSÓK, J: We tested other metal ions as well. It was found that Zn^{2+} was less toxic for fishes as compared to the Cu^{2+}. Namely, proteolytic enzyme activity and acetylcholinesterase activity were not so much inhibited than in the case of Cu^{2+}.

THEEDE, H: The effects observed occurred at relatively high Cu-concentrations. What is the relation to concentrations occurring in the field? What is the sensitivity of the enzyme system studied by you in comparison to the traditionally studied parameters as growth rate, reproduction, developmental speed, etc.?

NEMCSÓK, J: The 10 ppm $CuSO_4$ concentration is not so extreme, because fishes might accumulate pesticides in their organism in 1000 fold higher concentration as compared the concentration of a given pollutant occurring in the water (See: in another lecture of this Symposium; H.-Miklovics et al.:Accumulation and effect of heavy metals in the fish of Lake Balaton, p. 111 As to your second question, we did not make special comparisons, however, changes in the enzyme system reflect the effect of much shorter exposure time than the parameters you mentioned.

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GENERAL DISCUSSION

SALÁNKI,J: During the past days we had the opportunity to discuss the papers presented at this Symposium. There are, however, some general questions which arose repeatedly but remained unanswered or were discussed only outside of the lecture hall or have not been mentioned at all so far. At the end of the Symposium we can recall some of them.

Listening to the papers and considering the literature one finds a great deal of new information on heavy metal concentrations in various living organisms and organs from a wide variety of locations. However, in many cases the comparability is questionable due to different sampling techniques and measuring methods. One should find some solution to this problem and I wonder whether somebody has a good proposal. Another aspect is whether it is necessary to make measurements for a large number of organisms in each location, or it would be possible to restrict them to some indicator species or organs which are the best accumulators for one or the other heavy metal. A selected list of indicator organisms or tissues would be of great help in the environmental monitoring practice.

One of the aims of the IUBS Bio-indicator program is to work out recommendations for the biological monitoring of the sublethal effect of anthropogenic pollutants, among them, of heavy metals. The question is what could be the validity of functional methods in different geographical regions or under climatic circumstances? Certainly, exchange of experiences of different laboratories, control of testing methods under different

circumstances and discussion of the results at meetings like this may be of great importance in this respect.

The "mussel watch" seems to be a good biological monitoring system for some sort of pollution in marine ecosystems. It would be important to clarify, whether fresh water mussels could be used in the same way to control lake and river pollution. Our results suggest that this is a real possibility, but other organisms should be tested as well.

A further group of questions is concerned with the sublethal effect of heavy metals. Organisms can adapt to changes in the environment and the the presence of undesirable substances. However, we have no information to what extent vital functions of organisms are at the same time influenced. Laboratory experiments are usually carried out with much higher concentrations of pollutants than occur in the environment. We do not know what the reality is of the extrapolations of laboratory results to natural situations. To clarify this, extensive research should be done using the same species and long term exposure to low doses of pollutants. Nevertheless, the testing of the functional effect of heavy metals in laboratory conditions seems to me very important and I think such research should be stimulated and supported.

SHIBER,J: Suggested or recommended the following:
- indicator species used for pollution studies must be readily available in all the areas that are concerned with the standardization of work on pollution monitoring
- analysis of the same samples for heavy metals at least in two different laboratories for stronger validation of results
- verification of taxa (species) by expert taxonomists for experimental work on heavy metals
- application of strict laboratory experimental results to the "real" field situation where the real pollution situation may or may not exist.

WACHS,B: One main reason that there is no common watch organism for the control of freshwater contrary to the "mussel

watch" for sea waters will be that the chemisms and the ecosystems are too different due to their location. Strongly organic polluted rivers as well as not organic polluted waters can be contaminated with heavy metals and the local organisms are in most cases other species. If we will choose not only one but several plant species (i.e. Fontinalis, Cladophora, Ramunculus) we may have a way to control the water and to compare the results.

FOWLER,S.W: Sampling technique and great care during measuring procedure is extremely important. In case this is not considered, no reliable and comparable results will be collected. E.g. it is very important to investigate what is in the stomach of mussels used in "mussel watch", otherwise the results will not be correct.

It is important to include in any monitoring programme, international or national, a comprehensive intercalibration of analytical methods to ensure quality control of data. The best is if a laboratory or a group of scientists is responsible for the elaboration of the techniques and for the control of the validity of the measurements.

Besides field and laboratory experiments also "half-field" experiments are important, when organisms are planted from one place to another. The response of the organism can be evaluated as a result of the new circumstances (pollution, etc.).

WEIS,P: If an animal is tolerant to some unusual substance, it does not mean that there is no problem with this substance in the environment. On the contrary, tolerance means that there is a problem and tolerance is the reaction of the animal.

Field validation of laboratory experiments is very difficult, but extremely important in order to get a real picture on the effect of heavy metals and other anthropogenic substances.

LORCH,D: Worldwide pollution, not only with heavy metals, makes global monitoring of toxicant input a necessity. For this purpose the integrative capacities of organisms in biomonitoring should be made use of. A prerequisite is, that uniform or-

ganisms and standardized methods are employed. Thus intercalibration of the analytical procedures should be a first step. I personally, however, doubt if a single species with worldwide distribution and the necessary other requirements for biomonitoring can be found. Even if this were possible the great variation of external and biotic factors influencing heavy metal accumulation at the different sampling sites would prohibit direct evaluation and comparison of the metal concentrations found and their correlation with toxicant input. However it should be feasible to suggest a whole spectrum of organisms for cumulative biomonitoring i.e. algae, or higher macrophytes, where submerged species should be preferred. For short time monitoring (in the range of days to weeks) plant material from standardized laboratory cultures might be exposed in situ thus eliminating some of the biotic variations and ensuring homogenity in the monitoring organisms.

BRIX,H: Fresh water ecosystems are rather different and possibly the organisms which are useful in biomonitoring are accordingly also different. It seems better to find and investigate in each ecosystem the most characteristic and useful monitor organism and it is not necessary to look after some general one which would be suitable everywhere.

SALÁNKI,J: I do not think there is a need to make any specific conclusion at the end of the general discussion. There were good proposals but controversial views have still remained in some questions. Nevertheless, I am sure, listening to each other's opinion and argumentation everybody feels in which respect he or she is right or wrong, and can utilize this experience is his or her future work.

No doubt, both the results presented and the questions discussed enrich our knowledge and thinking and this is a good result of our meeting. Thank everybody for the participation and for the contribution to the success of the Symposium.

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